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The interrelationship of sleep, biologic clocks, neurotransmitters, gonadotropins and pubertal development
J.H. Check - Camden, NJ (USA)
Biologic clocks, sleep, and neurotransmitters play a key role in awakening the GnRH pulse generator and thus initiating puberty.

Reproductive Biology Section
Isolating sperm by selecting those with normal nuclear morphology prior to intracytoplasmic sperm injection (ICSI) does not provide better pregnancy rates compared to conventional ICSI in women with repeated conception failure with in vitro fertilization
J.H. Check, A. Bollendorf, D. Summers-Chase, W. Yuan, D. Horwath - Camden, NJ (USA)
Using high-powered magnification to isolate sperm with normal nuclei prior to ICSI provides no advantage over conventional ICSI.

Intracytoplasmic sperm injection allows normal pregnancy rates for males ≥ 40 with low hypoosmotic swelling test scores even when complicated by very low motility percentage
J.H. Check, A. Tubman, C. Wilson - Camden, NJ (USA)
Low percentage motility has no additional negative effect on sperm with low HOS scores when used for intracytoplasmic sperm injection.

Pregnancy rates following the exclusive transfer of twice frozen twice thawed embryos using a modified slow cool cryopreservation technique
The exclusive transfer of twice-frozen twice-thawed embryos resulted in a live-delivered pregnancy rate of 18%.

Embryo apoptosis may be a significant contributing factor in addition to aneuploidy inhibiting live deliveries once a woman reaches age 45
J.H. Check, S. Burgos, B. Slovis, C. Wilson - Camden, NJ (USA)
Embryo apoptosis is the most likely main factor for low pregnancy rates in women ≥ 45 years even with normal oocyte reserve.

Adding luteinizing hormone to follicle stimulating hormone from day 3-5 improves pregnancy outcome in normal but not poor responders using gonadotropin releasing hormone antagonists
The addition of LH to FSH stimulation improves pregnancy rates even in women using gonadotropin releasing hormone antagonists.

The effect of diminished oocyte reserve in younger women (age ≤ 37) on pregnancy rates in natural cycles
J.H. Check, J. Liss - Camden, NJ (USA)
Women with marked oocyte depletion are half as likely to achieve a pregnancy compared to women with normal oocyte reserve.

Younger women with diminished oocyte reserve are not more prone to meiosis errors leading to spontaneous abortion than their age peers with normal oocyte reserve
B.H. Slovis, J.H. Check - Camden, NJ (USA)
Advancing age rather than the degree of oocyte depletion is associated with a higher risk of miscarriage.
Intrauterine insemination (IUI) does not improve pregnancy rates in infertile couples where semen parameters are normal and postcoital tests are adequate

J.H. Check, J. Liss, A. Bollendorf - Camden, NJ (USA)
Performing a time intrauterine insemination for women with normal post-coital tests and male partners with normal spermiograms does not improve chance of pregnancy.

Low hypo-osmotic swelling tests correlate with low percent motility and age of the male

A. Tubman, J.H. Check, A. Bollendorf, C. Wilson - Camden, NJ (USA)
Males with low HOS test scores are more likely to be of an older age and sperm motility defects are more common.

Effect of triple line vs isoechogenic endometrial texture on pregnancy outcome following embryo transfer according to use of controlled ovarian stimulation (COH) or estrogen/progesterone replacement

J.H. Check, C. Dietterich, J.K. Choe, R. Cohen - Camden, NJ (USA)
The oral supplementation with antioxidant agents containing alpha lipoic acid may help to prevent postmenopausal bone loss.

General Section

Frequency of endometriosis and adenomyosis in patients with leiomyomas, gynecologic premalignant, and malignant neoplasias

R.S. Nomelini, F.A. Ferreira, R.C. Borges, S.J. Adad, E.F.C. Murta - Uberaba, MG, Brazil
The association between gynecological neoplasms, endometriosis, and adenomyosis in women who underwent surgical treatment for gynecological cancer and uterine leiomyoma was studied.

Placental apoptosis in preeclampsia, intrauterine growth retardation and HELLP syndrome: An immunohistochemical study with caspase-3 and bcl-2

U. Cali, S. Cavkaytar, L. Sirvan, N. Danisman - Ankara, Turkey
Apoptotic marker caspase-3 is significantly increased in the villous trophoblast of patients with preeclampsia, HELLP syndrome, and IUGR indicating increased placental apoptosis.

Obstetric outcome in adolescence: a single centre experience over seven years

D. Kellartzis, D. Tsolakidis, T. Mikos, D. Vavilis, V. Tzeveleakis, G. Tampakoudis, B. Tarlatzis - Thessaloniki, Greece
Retrospective review of adolescent pregnancies: a 7-year experience.

The impact of socio-economic, lifestyle habits, and obesity in developing of pregnancy-induced hypertension in fast-growing country: global comparisons

A. Bener, N.M. Saleh - Doha, Qatar
The risk factors influencing pregnancy-induced hypertension are considered in a population of fast-growing country.

Arterial hypertension and female sexual dysfunction in postmenopausal women

Sexual function in post-menopausal women may be impaired by hypertension mainly when no pharmacological treatment is administered.

Gestational hypertension risk evaluation based on epidemiological, biochemical, and hemodynamic factors

L. Yang, W. Zhang, L. Zhang, S. Zhang, Y. Yang, Q. Wang, J. Shao, G. Chen, Y. Wang - Beijing, China
The knowledge of gestational hypertension risk factors is important for early treatment that improves the quality of perinatal care.

Relevance of anti-Müllerian hormone on in vitro fertilization outcome

E. Celik, E. Bastu, O. Dural, C. Yasa, F. Buyru - Istanbul, Turkey
Serum and follicular anti-Müllerian hormone (AMH) concentrations were investigated. Positive correlation between serum AMH and ovarian reserve was observed.

Doppler parameters of maternal renal blood flow in normal pregnancy

V. Mandic Markovic, Z. Mikovic, M. Djukic, S. Simic Ogrizovic, M. Vasiljevic - Belgrade, Serbia
Maternal renal resistance indices do not change in normal pregnancy as a result of physiological changes of glomerular filtration rate.
Comparison of transvaginal 3D sonohysterography with outpatient hysteroscopy in the evaluation of abnormal uterine bleeding
Prospective randomized controlled study, which aims at comparing transvaginal 3D sonohysterography with outpatient hysteroscopy accuracy, procedure time, and discomfort.

Factors affecting completion of laparoscopic myomectomy
E.H. Yoo, S.K. Lee - Seoul, KOREA
The completion of laparoscopic myomectomy without unintended surgery is conditioned by the surgical experience and the knowledge of the risks.

How to prevent the complications caused by the changes of pelvic anatomical relationship after gynecological surgery?
Xu Tianmin, Chang Weiqin, Cui Manhua, Si Lihui, Wei Tianshu - Chanchun City, CHINA
Some diseases caused by change of pelvic anatomical relationship after gynecological surgeries, as well as their prevention and treatment are discussed.

Bilateral hypogastric artery ligation in emergency setting for intractable postpartum hemorrhage: a secondary care center experience
F.K. Boyanukalin, H. Boyar, H. Gormus, A.I. Aral, N. Boyar - Ankara, TURKEY
Hypogastric artery ligation should be kept in consideration as a fertility-saving procedure during intractable postpartum hemorrhage.

Loss of heterozygosity in the fragile histidine triad (FHIT) locus and expression analysis of FHIT protein in patients with breast disorders
Loss of heterozygosity in the fragile histidine triad gene may be related to menopause in women with breast disorders.

The value of negative chlamydia trachomatis antibody in prediction of normal tubes in infertile women
Z. Raoofi, M. Barchinegad, L. Haghighi - Tehran, IRAN
The sensitivity and specificity of the negative predictive value of chlamydia trachomatis antibodies is 100%.

Evaluation of low-dose letrozole addition to ovulation induction in IVF
C. Yasa, E. Bastu, O. Dural, E. Celik, B. Ergun - Istanbul, TURKEY
Addition of letrozole in IVF treatment may reduce the dose of gonadotropin administered. However, clinical practice remains questionable due to no evident positive effect on pregnancy rates.

Lentivirus vectors mediated eGFP transfected into rat ovary in vivo
W. Jidong, L. Shuang, P. Hongjuan, Y. Zhenwei, M. Xiaohui - Chongqing City, CHINA
Lentivirus vectors mediated gene could express stable in a form at long-term in rat ovary.

Practical biometric ratios of first-trimester screening
R.N. Ergin, M. Yayla, A.S. Ergin - Istanbul, TURKEY
The ratios CRL/BPD, AC/BPD, and CRL/AC seem to be helpful in checking abnormal or mistake measurements.

Immunohistochemical study of Inhibin A and B expression in placentas from normal and pathological gestations
A. Kondi-Pafiti, C. Grigoriadis, D. Samiotaki, A. Filippidou-Giannopoulou, C. Kleanthis, D. Hassiakos - Athens, GREECE
Immunohistochemical comparative study of Inhibin-A and B expression, in placentas from normal and pathological pregnancies.

Ultrasound parameters and L/S ratio in prediction of perinatal outcome in term-growth restricted newborns
I. Babovic, Z. Radojicic, S. Plesinac, S. Aksam - Belgrade, SERBIA
Lecithin/sphingomyelin ratio and biophysical profile are good predictors of perinatal outcomes in IUGR newborns.

Symptomatic Shigella sonnei urinary tract infection in pregnancy
S. Baka, A. Spathi, I. Tsouma, E. Kouskouni - Athens, GREECE
Shigella sonnei can cause UTI during pregnancy in the absence of predisposing factors or an apparent source of infection.
Single dose epidural morphine instead of patient-controlled epidural analgesia in the second day of Cesarean section; an easy method for the pain relief of a new mother

A. Bilir - Eskisehir, TURKEY

Pain management is important after cesarean section. Single-dose epidural morphine provides satisfactory pain control in post-operative 24-48 hours.

The efficacy of intrauterine versus oral progestin for the treatment of endometrial hyperplasia. A prospective randomized comparative study

K. Dolapcioğlu, A. Boz, A. Baloglu - Hatay, TURKEY

Evaluation and comparison of the long-term outcomes of oral progesterone and LNG-IUD therapies applied for the same length of time against hyperplasia without atypia.

Ondansetron or metoclopromide? Which is more effective in severe nausea and vomiting of pregnancy? A randomized trial double-blind study

M. Kashifard, Z. Basirat, M. Kashifard, M. Golsorkhtabaramiri, A. Moghaddamnia - Babol, IRAN

Ondansetron has more favorable effect in controlling severe vomiting than metoclopromide.

Liquid based cytology and HPV DNA testing in a Greek population compared to colposcopy and histology


Evaluation of the accuracy of cytological findings from a Greek observational population sample and association with DNA testing, colposcopy, and histology.

The role of mini laparotomy in patients with uterine myomas

D. Zygouris, G. Androutsopoulos, C. Grigoriadis, E. Tetzakis - Rion, GREECE

The role of mini laparotomy in patients with subserosal and/or intramural uterine myomas.

Ankaferd blood stopper in episiotomy repair

E.G. Yapar Eyi, Y. Engin-Üstün, M. Kaba, L. Mollamahmutoğlu - Ankara, TURKEY

Topical application of Ankaferd, a natural hemostatic agent, is useful in episiotomy repair.

Type of delivery and self-reported postpartum symptoms among Iranian women

M. Nikpour, M.A. Delavar, Z. Abedian - Babol, IRAN

Postpartum course is conditioned by the quality of labour and delivery.

Comparison of HbA1c levels in obese and non-obese polycystic ovarian patients

A.N. Unluer, R.B. Findik, N. Sevinc, J. Karakaya - Ankara, TURKEY

HbA1c level gives an indication to prevent cardiovascular risks in polycystic ovarian patients, both obese and non-obese.

Administration of lopinavir/ritonavir association during rat pregnancy: maternal and fetal effects

L. Kulay Jr., C.C. Hagemann, M.U. Nakamura, R.S. Simões, A. Moreira de Carvalho, R.M. Oliveira-Filho, S. Espiridião - São Paulo (SP), BRAZIL

Lopinavir/ritonavir treatment during rat pregnancy can cause maternal death; however, no fetal toxicity was detected.

Surgical repair of a complicated urethro-vaginal fistula: case report and review of the literature

C. Grigoriadis, P. Bakas, A. Liapis - Athens, GREECE

A case of complicated urethro-vaginal fistula successfully treated after Martius flap surgery together with review of literature are reported.

Misoprostol for labor induction in the second trimester in a woman with previous three cesarean deliveries and an intrauterine death of an anencephaly

A.A. Rouzi - Jeddah, SAUDI ARABIA

Misoprostol can avoid hysterotomy for induction of labor in case of intrauterine death at the second trimester.

Repeated term pregnancies in a young patient with pelvic organ prolapse

Ş. Özyer, Ö. Uzunlar, A. Payaşı, C. Toğrul, M. Beşli, N. Danışman - Ankara, TURKEY

Cesarean section is the elective choice for a plurigravida patient affected by edema and necrosis of the prolapsed cervix.
Ultrasound diagnosis of recurring Jeune’s syndrome: a case report
E.N. Kontomanolis, E. Markopoulou, P. Pinidis, A. Georgiadis, S. Kokkoris, V. Limperis - Alexandroupolis, GREECE
A case report of two successive pregnancies with Jeune’s Syndrome, which is a rare skeletal dysplasia associated with multiple organ anomalies is presented.

Benign pelvic metastatic leiomyoma: case report
H. Wei, Y. Liu, H. Sun, F. Qian, G. Li - Beijing, CHINA
A case of pelvic BML, who underwent surgical resection four years ago and recovered well is reported.

Pyomyoma after dilatation and curettage for missed abortion
F.G. Ugurlucan, A.C. Iyibozkurt, S. Sen, O. Kuru, S. Berkman - Istanbul, TURKEY
Broad spectrum antibiotics and myomectomy was the therapy for a patient with pyomyoma after curettage for missed abortion.

The management of fusion of the labia minora pudendi in adult women using a radiosurgical knife
M. Prorocic, M. Vasiljevic, L. Tasic, O. Džatić, S. Brankovic - Belgrade, SERBIA
A successful case of knife radiosurgical procedure in patients with fusion of the pudenda labia minora is reported.

Spontaneous rupture of uterine varices in third trimester pregnancy: an unexpected cause of hemoperitoneum. A case report and literature review
A rare case of hemoperitoneum during the third trimester of pregnancy due to varices spontaneous rupture is reported.

Laparoscopic myomectomy of a giant myoma
A. Kavallaris, D. Zygouris, N. Chalvatzas, E. Terzakis - Athens, GREECE
The case of an infertile woman with a 18-cm uterine myoma which was laparoscopically removed is presented.
The interrelationship of sleep, biologic clocks, neurotransmitters, gonadotropins and pubertal development

J.H. Check

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Summary

Purpose: To evaluate the influence of sleep on early pre-pubertal and pubertal development and to explore the importance of circadian rhythms and gonadotropin secretion. Methods: Mechanisms of suppression and turning on of the hypothalamic gonadotropin releasing hormone (GnRH) pulse generator at different times in development are evaluated. Furthermore, the influence of neurotransmitters in controlling pubertal development is also considered. Results: By the end of the first year, certain genes are activated that cause marked sensitivity of the GnRH pulse generator to negative feedback of circulating sex steroid. Furthermore, a central nervous system mechanism contributes greatly to the juvenile pause. Biologic clocks help to turn on the gonadostat and loss of negative feedback to sex steroid. This occurs during the sleeping hours. Equally important is to neutralize the neurotransmitter gamma amino butyric acid (GABA) which is the main central nervous system inhibitor. Conclusions: Pubertal development is a complex process requiring the activation of certain genes which activate biologic clocks. This results in the increased secretion of certain neurotransmitters, for example, leptin and kisseptin, which are very important in awakening the GnRH pulse generator. Suppression of the inhibitory neurotransmitter GABA is equally important.

Key words: Biologic clocks; Sleep; Neurotransmitters; Gene activation; Gonadotropin releasing hormone; Pulse generator.

Gonadotropins and sex steroids in the fetus neonates and infants

The various hypothalamic factors responsible for the production and the release of various pituitary hormones are not fully developed at birth [1]. Minutes after birth in the male, neonate serum luteinizing hormone (LH) significantly increases in the serum which causes an increase in serum testosterone within three hours that persists for 12 hours or more [2]. This very early LH secretion is not found in the female neonate [2].

Within the first few days of life, differences in follicle stimulating hormone (FSH) and LH are found between male and female infants with both sexes displaying pulsatile patterns, but the amplitude of FSH pulses is much greater in the female infant [1]. The difference between sexes may be related to the effect of the early testosterone secretion of the male from 11 - 24 weeks related to hypothalamic development [1].

Toward birth, a drop in serum FSH and LH occurs in relation to the development of estrogen, testosterone, and progesterone receptors in the hypothalamus, thus allowing negative feedback from these sex steroids [3, 4]. Inhibin can also exhibit a negative feedback in late gestation [5]. Actually the change from high levels of sera FSH and LH begins at mid-gestation with increasing sensitivity to the negative effect of sex steroids on hypothalamic GnRH secretion with the formation of sex steroid receptors. The placenta is the source of estrogen and progesterone which exert the negative feedback effect and testosterone from the fetal testes from mid-gestation to late gestation [5, 6].

Differences in the pulsatility and concentration of FSH and LH in males vs female neonates

The aforementioned rise in LH shortly after birth not only leads to a rise in serum testosterone, but high LH leads to a proliferation of sertoli cells and spermatogonia [2, 7]. Furthermore, more Leydig cells are produced leading to an increase in serum testosterone during the first few months of life [8].

The sensitivity of hypothalamic sex steroid receptors to negative feedback reaches a maximum by six months of age in the male. Thus the gonadotropins are maximally suppressed by six months and stay suppressed until puberty [8].

The gonadotropin patterns are somewhat different in the female neonate and infant. FSH pulse amplitude is much greater in the female infant. This leads to a rise in estradiol in the first 1.5 years of life [9]. The peak negative sensitivity of sex steroids on gonadotropins does not occur until two to three years of age in the female when the gonadotropins drop to very low levels which remain low until puberty [10]. Thus the inhibition of the GnRH pulse generator and thus the suppression of GnRH from the hypothalamus does not occur until late infancy or early childhood in females and even earlier in males.
Pubertal development – influence of genetics

Genetic factors (especially polygenic) are estimated to be 50 - 80% responsible for determining the timing of puberty when the GnRH pulse generator loses its sensitivity to the negative feedback effects of sex steroids [11, 12]. These genes control the development of the GnRH pulse generator through their effect of increasing stimulatory factors, for example, glutamate and kisspeptin, and decreasing inhibitory factors such as GABA and opioids [12]. The mechanism of delivering these stimulatory and inhibitory factors is through transsynaptic and glial-neuronal communications [12]. An example of a pathological state related to polygenic control of onset of puberty is constitutional delayed pubescence; however the exact gene loci for this polygenic influence on onset of puberty has not been completely identified.

Pubertal development – influence of leptin

Leptin is a protein resembling cytokines which is predominantly made by adipose tissue [13, 14]. It resembles cytokines and, in fact, its receptor is a member of the gp family of cytokines receptors [15]. Leptin acts on the hypothalamus to cause appetite satiety and thus plays a key role in metabolism and weight control [16, 17].

A mutant leptin gene in a group of very obese mice known as ob / ob mice was found to be etiologic for their obesity [18]. These mice have also been found to have hypogonadotropic hypogonadism [19, 20].

Some have considered leptin as the possible main peripheral trigger to the central nervous system “awakening” the hypothalamic pulse generator [21, 22]. Indeed leptin is secreted in a pulsatile manner [23]. Similar to gonadotropins at puberty leptin has a diurnal pattern with the highest levels at night and the lowest in the morning [16, 24]. Most of the studies favor a permissive role for leptin rather than leptin being the trigger for puberty [25-27]. It is believed that its main role is to signal the GnRH pulse generator that a critical energy store has been achieved [21]. The data actually favor that rather than leptin stimulating GnRH, it is in fact, the increase in GnRH pulsatility at puberty that causes rise in serum leptin [28].

Thus previous observations have found an earlier onset of menarche in moderately obese girls [29]. Similarly low weight states, such as anorexia nervosa or a decrease in body fat without low weight as in strenuous exercise has been associated with a delay in menarche [30-34]. With the discovery of the correlation of serum leptin levels with adipose mass and percentage of body fat [26, 27], at first it seemed reasonable to consider leptin as the initiating agent for puberty [26, 27]. However, as stated above, its secretion plays a role in the hypothalamic secretion of GnRH, but it may be more involved in enabling the hypothalamus and suprahypothalamic factors involved in the manner of GnRH secretion knowing that there is sufficient energy available to continue the pattern of pulsatile GnRH secretion that was originally initiated by other factors [28].

The permissive role of leptin cannot be overlooked although it maybe not the trigger; the ob / ob mice have increased body fat but because of mutated leptin receptors have hypogonadotropic hypogonadism [19, 35]. Actual analogous mutations have been found in either leptin or in the leptin receptor leading to marked obesity, and hypogonadotropic hypogonadism in humans [36, 37]. Evaluation of various pathological states, such as constitutional delayed pubescence and several genetic disorders leading to severe leptin deficiency have led to the conclusion that a critical level of leptin and a leptin signal are required to attain puberty (permissive role), but a rise in leptin is not needed to trigger the hypothalamic GnRH pattern needed to initiate puberty [27, 38].

Inhibitory effect on pubertal development of the central nervous system (CNS) after infancy

The intrinsic CNS inhibitory effect lasts for approximately a decade of life. As mentioned, there is an increased amount of LH and FSH in infants. However, shortly after infancy, there is a suppression of GnRH activity leading to diminished FSH and LH secretion. Certainly an important part of the low gonadotropin secretion is related to exquisite sensitivity to negative feedback of sex steroids.

Gonadal dysgenesis in Turner’s syndrome is a classic example of the influence of negative feedback effect of sex steroids, but also the presence of a central gonadotropin inhibitory mechanism and their ontogeny [39, 40]. Related to the gonadal state (and thus even less sex steroids made by the ovaries than the normal infant), baseline sera FSH and LH levels are higher in infant females with Turner’s syndrome than normal gonadal infant females [39, 40]. Furthermore, infant females with Turner’s syndrome show an exaggerated response to exogenous GnRH compared to normal infant females [39, 40].

However, the influence of the CNS inhibitory effect is evident even in females with gonadal dysgenesis because the mean gonadotropin levels drop to similar values, as their age peers with normal ovaries between the ages of 4 - 10 years [39, 40]. Since the inhibitory mechanism of the GnRH pulse generator occurs in agonal children who do not make sex steroids, it is clear that this CNS inhibitory mechanism is independent of the sex steroid mechanism of negative feedback.

As puberty approaches the CNS inhibitory mechanism which has been dominant since three to four years of age gradually loses its initial influence during nighttime sleep [1], but also the GnRH pulse generator becomes less sensitive to
the negative effects of sex steroids [1]. Nevertheless in the post-pubertal individual, whereas the CNS inhibitory mechanism seems to be completely eradicated, there still remains some sensitivity to the negative effects of sex steroids, and only the set point has been raised. The end of the so-called juvenile pause is initially shown by an increase in LH pulse amplitude during the early hours of sleep [41, 42].

Neurotransmitter and other potential influencing factors on the intrinsic CNS inhibitory mechanism: GABA

One of the most important inhibitory neurotransmitters in the brain is GABA. Most studies support GABA, which is generated by interneurons, as the intrinsic CNS inhibitor of the GnRH pulse generator during the juvenile phase before puberty [41-45].

GABA may actually have an excitatory effect on synaptic transmission in the post-natal period contributing to the higher basal gonadotropin levels in the post-natal period and increase intracellular calcium concentration [46, 47]. A developmental switch from GABA ergic excitatory to inhibitory states is most likely responsible for the diminished GnRH pulse generator activity in childhood [47].

The onset of puberty in the rhesus monkey (and probably in humans) is characterized by the decrease in GABA ergic and neuropeptide Y inhibitor of the hypothalamic GnRH pulse generator [48]. It is also characterized by the increased release of glutamate, which is considered to be the major excitatory amino acid neurotransmitter in the hypothalamic [49]. Of the two mechanisms, inhibition of the GABA ergic inhibitor of the GnRH pulse generator, is considered the more important event in the initiation of puberty [48].

Another excitatory neuropeptide may be N-methyl-d-aspartate (NMDA) which has been found to stimulate LH release in prepubertal and adult rhesus monkeys [50, 51]. N-methyl-d-aspartate has been found to induce the release of GnRH from the hypothalamus and in fact, one can cause prepubertal rhesus monkeys to enter puberty earlier by repeated infusions of NMDA [48].

The importance of kisspeptins

The KISS-1 gene is a human metastasis suppressor gene and KISS-1 mRNA is found in several tissues in the body, but in the brain it is mainly found in the hypothalamus and basal ganglion [52]. The secreted product of the KISS-1 gene is a 54-amino acid peptide called Kisspeptin (metastin is another name). The receptor for kisspeptin is a G protein known as GPR54 and is similarly found predominantly in the hypothalamus and basal ganglia. There are data suggesting that KISS-1 signaling through the GPR54 receptor of the primate and human hypothalamus may be activated at the end of the juvenile pause. This gene activation thus may play an important role in the turning on of the GnRH pulse activator at the initiation of puberty [53].

Ablating the pulsatility of GnRH by continuous infusion of kisspeptin suppresses GnRH release and subsequent secretion from the pituitary of LH and FSH. Similarly, continuous infusion of kisspeptin decreases the response of the gonadotropin cells to boluses of kisspeptin by down-regulation of the GRP54 receptors [54]. Kisspeptin-GPR54 signaling seems to play a critical part in the initiation and maintenance of puberty [55-57].

Summary of the relationship of neurotransmitters and initiation of puberty

Thus to summarize these events, the GABA ergic neuronal network with its neurotransmitter GABA is the main inhibiting transmitter in the hypothalamus and is responsible for the juvenile pause during the pre-pubertal years. The waning of GABA inhibitor of the GnRH pulse generator allows its reactivation. Kisspeptin excitant amino acids help to reactivate the GnRH pulse generator. The activation of the kisspeptin amino acids is enhanced by excitatory neuropeptides, such as: glutamate and N-methyl-d-aspartate (NMDA) and by nitric oxide, noradrenergic pathways, and growth peptides. Furthermore the increase in sex steroids exerts a positive effect on some of the kisspeptin excitatory amino acids and other neuroexcitatory factors.

However, independent of sex steroids, there is an increase in KISS-1 mRNA expression in kisspeptinergic neurons in the medical basal hypothalamus which allows the secretion of kisspeptins. As a consequence of kisspeptins binding to the GPR54 receptor on the surface of the GnRH neurons, this sequence increases the amplitude of GnRH pulses and also, but to a lesser degree, increases the frequency of GnRH pulses. The increase amplitude and frequency of GnRH pulses then increases the amplitude and frequency of LH and FSH pulses and this causes an increase is sex steroid production by the ovary or testes leading to pubertal changes. The mechanism is not yet completely understood. Though it is clear that puberty results from the removal of GABA inhibiter and reactivation of the GnRH pulse generator, it is unclear what event triggers this transition.

Biological clocks and sleep

Critical changes in the secretion of various endocrine hormones frequently have cyclical or periodic changes. These periodic or cyclical changes are independent of the environment. The biologic clock that drives these rhythms are mainly under the control of the nervous system.
Though these biologic clocks are independent of the environment, they are coordinated by external signals. One of these signals is light - dark changes and another somewhat-related signal is the ratio between the length of day and night [58-60]. These light - dark signals are known as Zeitgeber or "time givers". Light-dark signals influence the most diurnal rhythms (approximately a day). The 28-day menstrual cycle is referred to as infradian (longer than a day) rhythm.

Most endocrine rhythms are circadian. The secretion of growth hormone and prolactin (PRL) in humans is maximal shortly after the onset of sleep. For adreno corticotropic hormone (ACTH), the secretion begins about 4:00 a.m. and peaks at 7:00 a.m. As previously mentioned, gonadotropin secretion in adolescents is increased at night and characterized by rapid high amplitude pulsations. This phenomenon stops, however, when full maturity is reached.

The suprachiasmatic nuclei, as the name implies, lies above the optic chiasm as paired nuclei in the hypothalamus and is considered to be the area responsible for most of the circadian rhythms. Isolated cells of the suprachiasmatic nucleus have intrinsic capacity to oscillate in a circadian pattern [61]. The suprachiasmatic nucleus is rich in neuropeptides including neuropeptide Y, somatostatin, and vasoactive intestinal peptide.

The majority of the suprachiasmatic neuronal activity terminates in the dorsal medial nucleus of the hypothalamus. Among other functions through this pathway, the suprachiasmatic nucleus produces circadian rhythms involved in sleep and arousal [60].

The suprachiasmatic nucleus has been found to possess MT1 and MT2 melatonin receptors [62]. Thus the suprachiasmatic nucleus can respond to the secretion of melatonin from the pineal gland. The suprachiasmatic nucleus projects to the pineal gland through the autonomic nervous system and the paraventricular nucleus. Noradrenergic sympathetic nerve terminals are critical regulators of melatonin production and release of melatonin from the pineal gland. The retina directly innervates the suprachiasmatic nucleus through the retino-hypothalamic tract. In the absence of light, the pineal gland rhythms persist but are not entrained to the external light-dark cycle.

The main substance secreted by the pineal gland is melatonin. Melatonin is very important in the regulation of many circadian rhythms. One of the most important signals is darkness. In many mammalian species, melatonin levels are highest during darkness and suddenly plummet in the presence of light.

In certain animals, melatonin plays a critical role in the initiation of puberty and in the control of gonadotropin secretion. Removal of the pineal gland in some species leads to precocious puberty. Male rats that are kept in constant darkness or are made to become blind will develop testicular atrophy. However, this can be prevented by removing the pineal gland, thus suggesting the mechanism of gonadotropin suppression is through melatonin. This mechanism can play a role in breeding of some species at certain times to allow a favorable time for delivery.

The pineal gland probably does not have a significant effect in the development of puberty or gonadotropin secretion in humans [63]. As mentioned in rats, creation of blindness by enucleation show testicular atrophy and reduced testosterone secretion. Yet in humans, early onset of menarche has been described in studies of blind women [64]. It has to be realized that certain species depend more on melatonin controlling and entraining circadian rhythms than the human species, and some species are more sensitive to the effects of light - dark on melatonin synthesis. It takes much more light in humans to produce an equivalent nocturnal suppression of melatonin compared to rodents [65].

Regardless of the species, the mechanism of melatonin control seems to be similar. Melatonin mediates its effects by interacting predominantly with an MT1 receptor (which is from the family of G proteins – couples receptors) and to a lesser degree with MT2 receptors (from the same family). Its main action is to inhibit the activity of neurons in the suprachiasmatic nucleus and thus acts to inhibit the master mammalian circadian pacemaker in the brain [66-68]. It is not completely clear, but it is known that melatonin plays some role (and it may vary according to species) in the effect of light to induce certain circadian phase shifts.

Actually, melatonin therapy has been used in humans to treat certain circadian-based sleep disorders by resetting circadian rhythms and causing some phase shifting [69]. It has been used with limited efficacy as a sleep aid in humans who do not have a disturbance in their circadian rhythms.

### Peripheral extra-SCN oscillators: biologic clocks and gonadotropin secretion

As mentioned, in the early 1970’s, the SCN based on extensive experimentation was considered the master circadian clock in mammals [70, 71]. However, with the discovery of clock genes, it became clear that there are multiple peripheral oscillators throughout the body [72-74].

Nakao et al. recently discovered that clock genes “per 2” and “per 3”, as well as “clock” and “bam/1” are expressed in preovulatory follicles [75]. Nakao et al. found that “per 2” and “per 3” expression is only found in the largest follicle that is first in line to ovulate [75]. They were not found in the smaller follicles.

It has been well known that the dominant follicle is in charge of its own destiny, i.e., effects the control of the complex relationship of positive or negative feedback control of LH and FSH release from the pituitary, especially with certain timed cyclic events the sex steroids, as estrogen, inhibit LH release then change to now exert a positive effect on LH release. The study by Nakao strongly suggests that clock genes, and thus peripheral oscillations in the developing follicle itself, must play a role in circadian mechanisms involving gonadotropins and sex steroids [75].
Nakao et al. found a gene related to increased synthesis of progesterone called steroidogenic acute regulatory protein (STAR). The gene was found to demonstrate a 24-hour cycle in the largest follicle which coincided with the expression of “per 2”. Thus this study suggests that LH induction of progesterone synthesis is gated by a circadian rhythm in clock gene expression in the largest follicle that is ready to ovulate [75]. Nakao et al. demonstrated that the fifth flanking region of the STAR gene contains E-box enhancers which can bind to CLOCK / BMAL1 heterodimers to activate gene transcription (clock genes through a process of coordinated feedback between transcription and translation are able to produce an oscillation that controls many different circadian rhythms).

Thus the data from Nakao et al. strongly suggest that the brain is not solely in control of regulating the ovary and timing of the secretion of sex steroids. Instead, the dominant ovarian follicle and the brain seem to equally share as circadian clocks gating the timing of the LH surge and thus ovulation.

The interrelationship of sleep, biologic clocks, gonadotropin secretion, and pubertal development

Sleep and circadian rhythmicity (intrinsic effects of time of day, irrespective of the sleeping or waking state) interact to produce the overall temporal pattern of the majority of hormones [76]. Growth hormone, PRL, LH, and FSH are secreted in large amounts during sleep, whereas thyroid-stimulating hormone (TSH) and ACTH secretion are reduced during the first half of the sleep period [76].

Even before any physical changes associated with pubertal development in five to six year-old children, a diurnal rhythm of serum LH and FSH and testosterone is able to be detected using a very sensitive radioimmunoassay [42, 77, 78]. During the early and mid-pubertal stages, LH pulses show greater amplitude and frequency during sleep [78, 79]. LH pulses are not detected during the day time at this stage of puberty. In late puberty, there are LH surges detected even during the daytime, but the amplitude and frequency is still higher during sleep. Once adulthood is reached, there is no longer a difference between awakeness and sleep. In males, the higher LH during sleep leads to an increase of testosterone. This increased LH secretion during sleep may be in some part caused by the infradian (referring to longer than a day) awakening on turning on of a suppressed gene in the CNS. Furthermore, the increased pulsatility is in part related to diminished negative feedback effect on the hypothalamus during the night.

There are data suggesting that regulation of fetal circadian rhythms may be mediated by maternal circulatory melatonin [65]. In fact, the timing of the human circadian pacemaker can be altered by the administration of melatonin [65]. It is tempting in view of the well-known association of darkness and melatonin to consider that melatonin plays some role in the initial activation of the CNS aspect of the nocturnal awakening of the hypothalamic GnRH pulse generator in pre-pubertal children [65]. However, there are no data at present to substantiate this hypothesis.

It is clear however that once GnRH activity is initiated, the GnRH itself has a self-priming effect on the gonadotropins increasing their sensitivity, and thus enhanced secretion of sex steroids to GnRH. This is substantiated by the demonstration that pubertal children secrete more LH and FSH in response to exogenous GnRH compared to pre-pubertal children. It is the increased LH response to synthetic GnRH that is one of the earliest hormonal markers of the onset of puberty.

The development of the positive feedback effect of estrogen, which is needed to allow ovulation, is a relatively late event probably commencing in mid-puberty. Thus the removal of the exquisite sensitivity of the negative feedback effects coupled with positive stimulatory effects from the CNS to the GnRH pulse generator allows sufficient follicular development to permit sufficient antral progression to make sufficient estrogen (though the follicles are probably androgen dominant at this stage) to allow menarche and other changes associated with increased estrogen production.

However, there is insufficient secretion of estrogen level attained to induce a positive feedback effect on LH, leading to a surge that is able to advance meiosis of an oocyte to a metaphase two stage and cause sufficient receptor changes to change the follicle from androgen dominance to estrogen dominance. During this time of estrogen production from antral follicles, they do not reach the dominant follicle stage. Nevertheless the amount of estrogen produced allows endometrial proliferation and eventually breakthrough bleeding occurs heralding the menarche.

For the first two years of menarche, the large majority of menstrual periods which occur irregularly (and especially with widespread intervals shortly after menarche), are anovulatory and are related to breakthrough bleeding [80].

Over time, first commencing during sleep and then extending to the awakening hours, the GnRH amplitude and pulse frequency progressively increase. The increased GnRH stimulation sensitizes the gonadotropins of the pituitary to increase FSH outright from a given GnRH bolus. Eventually the level of FSH becomes sufficient to allow one of the antral follicles to become the dominant follicle. According to the most recent theory, the Zeitgeber or “time giver” or “gate keeper” that synthesizes infradian (refers to a rhythm longer then the circadian day, e.g., one month as seen with the LH surge) monthly LH surge are the clock genes (e.g., “clock” and “BAM/1” and “per 2” and “3”), which are activated at a certain stage of folliculogenesis in the follicle destined to ovulate. This follicle is now able to synchronize the complicated events of turning estradiol from an inhibitor of LH to a stimulator in a given ovulatory cycle.

Thus the timing of pubertal involves a “developmental clock”, a singular timer, or a series of timers [81]. Using the clock analogy, the alarm but not the clock itself is species specific [81]. Expression of the alarm but not the clock itself is determined by the integration of the alarm with multiple permissive clues. As an example, one would consider the

clock itself that determines the year that a person will activate the GnRH pulse generator, it is the permissive cues that ultimately determines the month in which one or more genes that control the regulation of GnRH secretion are activated [81].

Though sleep and darkness have significant effects on the secretion of several pituitary hormones and melatonin from the pineal gland, there does not seem to be much influence of the suprachiasmatic nuclei on gonadotropin secretion. Similarly in humans there does not appear to be much influence of melatonin on gonadotropin secretion. Teleologically there must be some reason why the initiating events preceding menarche involve the removal of inhibitory factor for the GnRH pulse generator during the sleeping hours, but it is not yet clear as to whether there is some benefit to allow gonadotropins to be produced at the same time as other pituitary trophic hormones. Finally, it is unclear as to how the sleeping hours help to remove the CNS inhibitory neurotransmitters and the sensitivity to negative feedback effect of sex steroids to allow the development of GnRH and thus LH and FSH pulsatility and increased secretion during sleeping hours.

References

The interrelationship of sleep, biologic clocks, neurotransmitters, gonadotropins and pubertal development


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Isolating sperm by selecting those with normal nuclear morphology prior to intracytoplasmic sperm injection (ICSI) does not provide better pregnancy rates compared to conventional ICSI in women with repeated conception failure with in vitro fertilization

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Summary

Purpose: To determine if isolation of sperm by nuclear characteristics using high magnification offers any advantage over the normal morphologic methods when performing intracytoplasmic sperm injection (ICSI) in refractory cases in which the male partner had an abnormal DNA fragmentation index. Materials and Methods: Women aged ≤ 39 with failure to have a successful conception after three consecutive embryo transfers whose male partner had a DNA fragmentation index > 30% were randomly assigned to isolation of sperm for ICSI by a new high magnification procedure to evaluate nuclear morphology and the usual method with normal magnification where nuclear characteristics were not seen. Women 40-43 were not randomized and were given the option after hearing pros and cons. Results: Overall the live delivered pregnancy rates were similar in the high vs normal magnification groups (33.3% vs 36.3%). Conclusions: Isolation of sperm with normal nuclei with high magnification does not offer any advantage over conventional IVF for refractory cases where the male partner has a high DNA fragmentation index.

Key words: DNA fragmentation index; Intracytoplasmic sperm injection; Nuclear morphology; IVF failure.

Introduction

Repeated failure to conceive despite the transfer of morphologically normal embryos could be related to: bad luck, poor transfer technique, an occult oocyte factor (possibly genetic), an occult male factor (possibly genetic), an occult immunological factor, an occult endometrial factor, or an adverse effect of controlled ovarian hyperstimulation on the endometrium.

Options to consider would be to either try another IVF-ET cycle with lower dosage of gonadotropins, purposely freeze all embryos and defer fresh embryo transfer (ET) in favor of a future frozen ET, use donor oocytes, use donor sperm, pretreat with intravenous immunoglobulin or use lymphocyte immunotherapy, or use a gestational carrier.

The cheapest of all new options if one is guessing would be to use donor sperm. However, if donor oocytes is the wrong guess then the couple is spending money on another expensive IVF-ET cycle that may fail again.

Is there any evidence that a sperm factor could allow fertilization of the egg with apparent development of normal morphologic embryos that have low implantation potential? Such a defect has been found in males with hypoosmotic swelling tests < 50% when testing their semen [1]. Embryos formed by conventional insemination with low HOS test scores fail to implant [2, 3]. However, intracytoplasmic sperm injection (ICSI) fully corrects the problem [4].

Two sperm tests that at least in some studies have suggested that abnormalities of the tests are associated with low embryo implantation potential despite ICSI are: the sperm chromatin structure assay (SCSA) with DNA fragmentation indices DFI > 30% [5] and a high percentage of sperm with abnormal nuclear morphology [6]. Though initial studies with sperm with abnormal SCSA tests found no live babies despite ICSI, subsequent studies did not confirm the absolute failure to conceive but suggested high DFI index might be associated with higher miscarriage rates [7]. Studies of sperm highly magnified to 6000x have suggested that in some cases failure to conceive despite multiple failed IVF-ICSI cycles was related to the fact that the majority of the sperm had abnormal nuclei which could not be determined with an ordinary microscope [6, 8]. The authors suggested that the problem could be corrected by isolating sperm with normal nuclei and then performing ICSI [8]. The objective of the present study was to evaluate the pregnancy rates with a fourth cycle of IVF-ET in couples where the male partner had a DFI score > 30% and no
live pregnancies had been achieved in three previous IVF-ET cycles where the “treated” group had IVF-ET performed with ICSI using high powered magnification to isolate sperm with normal nuclei. In contrast, the control group had IVF-ET with ICSI using a conventional microscope and injecting sperm with normal morphology as determined by strict criteria.

Materials and Methods

The male partner of couples with failure to have a successful pregnancy despite three previous embryo transfers (fresh or frozen) had their semen sent to Dr. Evenson’s laboratory in South Dakota to perform the SCSA test. Only couples where fertilization was by ICSI were selected. Those couples in whom the male partner had DFI scores > 30% and in whom the female partner was aged ≤ 39 were randomly assigned to have the next cycle performed with isolation of sperm using a high powered microscope to select one sperm with normal nuclear morphology vs conventional ICSI, where the sperm was selected with normal morphology based on strict criteria. Couples aged 40-43 were informed that there was only a theoretical possibility but no evidence to support the contention that there could be an association with abnormal nuclei and abnormal SCSA test. They were advised that it was possible that the prolonged exposure to polyvinylpyrrolidone to slow the sperm sufficiently to allow evaluation of the nucleus could possibly have an adverse effect on the sperm. Thus this older group was given the option to use high magnification or not.

Only couples providing informed consent were evaluated. Thus the randomization was only given to couples in whom the female partner was aged ≤ 39. Couples in whom the female partner was aged 40-43 were allowed to choose between high magnification ICSI or conventional ICSI.

For the fourth IVF cycle all women were purposely treated with low dosage gonadotropins starting at no more than 150 IU per day. Live delivery rates were then compared in the younger group randomly assigned to high magnification ICSI vs conventional ICSI (in the older group where the choice of high magnification ICSI was decided by the couples).

Some women, especially those with borderline endometrial thickness chose to purposely freeze their embryos. They were not counted in the evaluation of pregnancy rates per fresh embryo transfer.

Results

In the randomized group (age ≤ 39) there were 12 randomly assigned to high magnification ICSI and 12 to conventional ICSI. There were six women who had high magnification ICSI and had fresh embryo transfer of at least two embryos vs nine with conventional magnification ICSI. Three women in the high magnification ICSI group cancelled the retrieval because of an inadequate number of follicles and three women froze all embryos because of borderline endometrial thickness. Two women in the normal magnification ICSI group cancelled the cycle because of inadequate response and one froze all embryos.

There were three live healthy deliveries in both groups (50.0% vs 33.3%). For women age 40-43, there was one live delivery of six choosing high magnification ICSI (16.7%) vs one of two (50%) choosing conventional ICSI.

Combining both randomized treatment groups, the live delivery rate was 40.0% (6/15) in the 4th IVF cycle with failures in the previous three which is approximately the normal live delivery rate for this IVF center even for 1st and 2nd embryo transfer cycles. For women aged 40-43 combining both groups the live delivery rate was 25% (2/8) which is also similar to what is found in cycles one and two for this age group in this IVF center.

Combining both age groups there were four live deliveries in 12 fresh embryo transfers using high magnification ICSI with a live delivery rate per transfer of 33.3% vs four of 11 (36.3%) with conventional ICSI.

Conclusions

Based on these pregnancy rates achieved in the fourth IVF cycle of women failing to conceive after three IVF attempts, it can be concluded that if an abnormal DNA fragmentation index is associated with male infertility it seems to be correctable by ICSI.

Isolation of sperm with normal nuclei using high magnification does not seem to be a method that will markedly improve pregnancy rates in refractory IVF
Isolating sperm by selecting those with normal nuclear morphology prior to intracytoplasmic sperm injection (ICSI) does not etc. Since no other modifications other than using lower dosages of gonadotropins were performed in these couples, it appears that failure to conceive despite three previous failed IVF-ET with ICSI cycles is not usually related to an occult genetic defect in oocyte or sperm, or an occult endometrial or immunological factor. One possible contributing factor to previous IVF-ICSI failures was the adverse effect on the endometrium or embryos by controlled ovarian hyperstimulation since all of these couples were treated in the 4th cycle with lower dosages of gonadotropins [9-12]. However, it is possible that merely bad luck explains the previous failure.

References


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Intracytoplasmic sperm injection allows normal pregnancy rates for males ≥ 40 with low hypoosmotic swelling test scores even when complicated by very low motility percentage

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Summary

Purpose: To determine if the additional burden of low percentage motility reduces the chance that sperm with low hypoosmotic swelling (HOS) test scores will achieve a pregnancy following in vitro fertilization (IVF) with intracytoplasmic sperm injections (ICSI). Methods: Couples undergoing IVF-embryo transfer (ET) and ICSI for low HOS tests (< 50%) were retrospectively identified. The percentage motility was divided into deciles. Pregnancy rates were determined according to the deciles of motility. Results: No differences in clinical or live delivered pregnancy rates per transfer were found in even the very lowest percent motility category. Conclusions: The added compounding factor of low percentage motility added to sperm with low HOS test scores does not reduce the effectiveness of IVF with ICSI.

Key words: Hypoosmotic swelling test; Sperm motility; Intracytoplasmic sperm injection; In vitro fertilization-embryo transfer.

Introduction

Males with sperm with subnormal hypoosmotic swelling (HOS) tests (< 50%) are generally infertile [1]. Interestingly, the abnormality does not prevent fertilization of the oocytes or production of morphologically normal embryos. Instead the defect has been clearly demonstrated to inhibit embryo implantation [2, 3].

The defect seems to be related to a toxic factor that causes impairment to the functional integrity of the sperm membrane which forms the basis of the test to detect this abnormality (impairment of the sperm membrane to allow normal osmosis) [4]. Based on pregnancy rates approaching zero following the transfer of embryos derived from conventional oocyte insemination but normal pregnancy rates with embryos formed by intracytoplasmic sperm injection (ICSI), it is assumed that the etiology for such poor pregnancy rates is the transfer of this toxic factor from sperm to zona pellucida by the supernumerary sperm that attach [5]. A further assumption is that with the incorporation of the zona pellucida into the embryo membrane the toxic factor is transferred to the embryo membrane which impairs its function. A functionally intact embryo membrane is needed for implantation [4].

Some studies have found a correlation of low percent motility and low HOS scores though perfectly normal appearing sperm may also have this defect [6]. The objective of this study was to determine if the sperm of males of more advanced age with low HOS test scores would lead to lower pregnancy rates if complicated by low percentage of motility despite IVF with ICSI.

Materials and Methods

The study was limited to couples undergoing IVF-embryo transfer (IVF-ET) whose male partner was age 40 or above (higher chance of low HOS test score with male age ≥ 40). The women were all aged ≤ 39.9. The only couples selected were those where the male partner had an HOS test score < 50%.

The clinical and live delivered pregnancy rates were evaluated according to the percent with progressive motility at 10% intervals.

Results

With motility % < 10, 10-19, 20-29, 30-39, 40-49, and ≥ 50%, the clinical pregnancy rates were 33.3% (2/6), 25.0% (2/8), 40.0% (4/10), 17.4% (4/23), 37.5% (6/16), and 42.9% (6/14), respectively.

The live delivered pregnancy rates were 33.3%, 12.5%, 40.0%, 13.0%, 31.3%, and 35.7%, respectively.
Discussion

The co-existence of sperm with a low HOS test score with very poor motility does not influence pregnancy rates when performing IVF with ICSI as evidenced by the group with the lowest percentage of motility doing as well as the group with normal motility.

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Pregnancy rates following the exclusive transfer of twice frozen twice thawed embryos using a modified slow cool cryopreservation technique


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Summary

Purpose: To determine the pregnancy rate following the exclusive transfer of twice frozen twice thawed embryos. Materials and Methods: All day 3 transfers of exclusive twice frozen-twice thawed embryos were retrospectively identified over a 13-year time period. The cryopreservation technique used a simplified slow cool freezing protocol. Results: Survival rates were 83.3%. The live delivered pregnancy rate was 18.1% (15/83). The implantation rate was 13.1% (22/168). Conclusions: These data suggest that twice frozen twice thawed embryos should not be discarded but either transferred alone if they are the only embryos left, or mixed with frozen embryos that have never been thawed. Though the live delivered pregnancy rates are inferior to fresh embryo transfer the marked reduction in cost and avoidance of the risk of ovarian hyperstimulation justifies their transfer.

Key words: Twice frozen; Twice thawed; Slow cool; Frozen embryo transfer.

Introduction

To maximize the success of a given in vitro fertilization program it is essential to have a good cryopreservation program [1]. First time frozen embryos are generated in various ways: 1) purposely freezing all embryos because of risk of ovarian hyperstimulation, 2) purposely freezing all embryos because of inadequate endometrial thickness, 3) purposely freezing because of the consideration that previous failures to conceive despite IVF-ET were related to adverse effects of controlled ovarian hyperstimulation, 4) the presence of extra embryos that were not transferred on the fresh embryo transfer cycle.

When a group of cryopreserved embryos are thawed the best ones are selected for transfer based on embryo morphology (e.g., blastomere number, fragmentation, and symmetry). The main objective of this study was to determine if a group of cryopreserved embryos are thawed and the best ones are selected for transfer is it worth re-freezing the remainder not transferred for future use or should they be discarded. Thus this study was aimed to ascertain the pregnancy rate following the exclusive transfer of twice-frozen, twice-thawed de-selected embryos.

Materials and Methods

All first day 3 embryo transfers over a 13-year-period exclusively using twice frozen and twice thawed embryos were identified. The embryos were cryopreserved using a simplified slow cool freezing protocol which used an alcohol bath controlled rate freezer. The cryoprotectant, 1,2 propanediol, was removed in one step [2]. A mixture of embryos originally frozen at the 2 pronuclear (PN) and multi-cell stage were allowed.

Results

There were 83 transfers of twice frozen twice thawed embryos. The woman’s average age at cryopreservation was 32.9.

The clinical (ultrasound at 8 weeks), viable (live fetus at 12 weeks) and live delivered pregnancy rates were 20.5%, 19.3%, and 18.1%, respectively. The implantation rate was 13.1% (22/168).

The initial survival rate of the group of embryos eventually forming the twice frozen twice thawed group was 96.2% for 2PN embryos and 83.7% for multi-cell embryos. The survival rate at the time of the second thaw was 83.2%.

Discussion

The first reported case of a pregnancy following the exclusive transfer of twice frozen twice thawed embryos using a slow cool technique was published in 1996 [3]. This is the largest series of twice frozen twice thawed de-selected embryos to be evaluated.

Though the pregnancy rates may be only half as good as expected with fresh embryo transfers, the considerable financial savings and avoidance of the risk of ovarian hyperstimulation syndrome with another IVF-ET cycle makes the transfer of these embryos worthwhile.

There has been only one other publication of a smaller series of transfers of twice thawed twice frozen embryos (n = 36) but these embryos were cryopreserved using vitrification [4].
References


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Embryo apoptosis may be a significant contributing factor in addition to aneuploidy inhibiting live deliveries once a woman reaches age 45

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Introduction
Poor pregnancy rates with advanced reproductive age is predominately related to a strong tendency for meiosis I and meiosis II errors; however there may be a contribution of a tendency for embryo apoptosis.

The objective of this study was to determine the relative contribution of aneuploidy vs embryo apoptosis on failure to achieve a live delivery in women of advanced reproductive age following in vitro fertilization-embryo transfer (IVF-ET). Furthermore, the study would determine the confounding effect of adequate vs diminished oocyte reserve (DOR) in women ≥ 40 years of age.

Materials and Methods
Women undergoing IVF-ET aged ≥ 40 years were divided into four age ranges: 40-42, 43-44, 45-46, and 47-49. They were further stratified according to normal day 3 serum follicle-stimulating hormone (FSH) ≤ 11 mIU/ml vs >12 mIU/ml. Results: For women aged 40-42 years there were no differences in live delivery pregnancy rates in women with normal vs decreased egg reserve (DOR). There were no differences in live delivery pregnancy rates in women aged 40-42 years vs 43-44 years with normal oocyte reserve; however despite no differences in clinical pregnancy rates in women aged 43-44 years with normal vs DOR, the live delivery pregnancy rates were markedly lower in the group with DOR. In contrast, there were very low chemical pregnancy rates in women aged ≥ 45 years. Conclusions: As seen in younger women, there does not appear to be any increased risk of meiosis errors in women aged 40-42 years with DOR compared to women of the same age with normal reserve. Low pregnancy rates in women aged 43-44 years with DOR is related to meiosis errors. In contrast the very low chemical pregnancy rates found in women aged ≥ 45 years despite embryo transfer (ET) suggest embryo apoptosis is mostly responsible for poor pregnancy rates in this very advanced reproductive age group.

Results
The pregnancy rates according to age and day 3 serum FSH in women of advanced reproductive age is shown in Table 1. Combining both FSH groups, 31.5% of ETs in women aged 40-42 years resulted in a + β-hCG vs 36.0% for women aged 43-44 years vs 6.8% for women aged 45-49 years (p < 0.0001 comparing women aged 40-44 years vs ≥ 45 years, Fisher’s exact test).

The live DR was 16.3% for women aged 40-42 years vs 11.3% for women aged 43-44 years vs 1.7% for ages 45-49 years (p < 0.0001 Fisher’s exact test). Though the clinical pregnancy rates per transfer were almost identical in women aged 43-44 years with normal vs DOR, there was a large difference in the live DR per transfer (p = 0.0178, Fisher’s exact test).

Discussion
DOR did not effect the live DR in women aged 40-42 years. However, women aged 43-44 years with oocyte depletion had a much lower chance of a live delivery, mostly related to aneuploidy as evidenced by similar clinical pregnancy rates per transfers to those women aged 43-44 years with normal day 3 serum FSH.

For women aged ≥ 45 years the very low rate of attaining a positive beta hCG level suggests that embryo apoptosis is highly prevalent in this group. These data
Embryo apoptosis may be a significant contributing factor in addition to aneuploidy inhibiting live deliveries once a woman etc.

Support previous conclusions that oocytes from younger women with DOR are not of similar quality to women of older age with similar degree of oocyte depletion, but have a quality more similar to their age peers [1-3].

Although women with normal oocyte reserve are less likely at age 40-42 years to have a successful pregnancy related to an increased risk of aneuploidy compared to younger women with normal oocyte reserve, women in this age group with DOR are not more prone to meiosis errors compared to their age peers with normal reserve as evidenced by similar live DR. The data from the 40-42 year old group supports the contention that the very low pregnancy rates reported by many other studies of IVF in women with elevated day 3 FSH may be related to the use of the high-dosage gonadotropins leading to embryos that appear normal but do not implant [4, 5].

References


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Table 1. — Pregnancy rates (PRs) according to age and day 3 serum FSH in women of advanced reproductive age.

<table>
<thead>
<tr>
<th>Age at time of retrieval (years)</th>
<th>40-42</th>
<th>43-44</th>
<th>45-46</th>
<th>47-49</th>
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<td># transfers</td>
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<td>% w / positive β-hCG</td>
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<td>21.5</td>
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Table 1. — Pregnancy rates (PRs) according to age and day 3 serum FSH in women of advanced reproductive age.
Adding luteinizing hormone to follicle stimulating hormone from day 3-5 improves pregnancy outcome in normal but not poor responders using gonadotropin releasing hormone antagonists


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Summary

Purpose: To determine if the addition of luteinizing hormone (LH) to follicle stimulating hormone (FSH) stimulation for controlled ovarian hyperstimulation (COH) protocols using gonadotropin releasing hormone (GnRH) antagonists improves pregnancy rates following in vitro fertilization-embryo transfer (IVF-ET). Materials and Methods: All IVF-ET cycles using a GnRH antagonist were evaluated according to whether FSH was used exclusively or if LH was added. The cycles were further stratified according to age (≤ 39 and 40-42 years) and according to good responders (≥ five oocytes retrieved) or poor responders (≤ four oocytes). Results: Combining all data, a significantly higher clinical and live delivered pregnancy rates were found in those adding LH (34.7% and 25.8%) vs those taking all FSH (33.4% and 25.8%). The only subgroup not showing this effect was the women aged 40-42 years with diminished oocyte reserve. Conclusions: LH should be added not only to COH protocols using GnRH agonists but also those using GnRH antagonists.

Key words: Luteinizing hormone; Follicle stimulating hormone; Gonadotropin releasing hormone antagonist; In vitro fertilization-embryo transfer.

Introduction

The introduction of highly purified follicle stimulating hormone (FSH) products where the presence of luteinizing hormone (LH) was minimized, and the later introduction of recombinant DNA technology where a completely pure FSH product could be manufactured, made it clear that except in circumstances of hypogonadotropic hypogonadism ovulation induction can be achieved in anovulatory women with FSH alone. This led to a large number of subsequent studies to determine if pregnancy rates are similar, higher, or lower with exogenous FSH stimulation alone vs LH and FSH together both in in vitro fertilization-embryo transfer (IVF-ET) cycles and non-IVF cycles.

To ascertain small significant differences, a study with sufficient power would be needed and it is unlikely that any given IVF center’s prospective or retrospective data would be sufficiently large enough to demonstrate significant differences. Thus one method to gain sufficient power is to combine studies and perform a meta-analysis. One of the first meta-analyses performed evaluating this question was a Cochrane meta-analysis [1]. It found a clinical pregnancy rate of borderline significance in favor of LH/FSH combination in induction cycles and IVF-ET and thus recommended the use of the less expensive human menopausal gonadotropins (hMG) preparation [1].

Subsequently a larger Cochrane meta-analysis was published [2]. This study found a significantly higher ongoing pregnancy rate with highly purified hMG compared to recombinant FSH in women undergoing IVF-ET [2].

This meta-analysis led to the largest randomized controlled trial to date comparing menotrophin versus recombinant FSH in-vitro fertilization trial (MERIT). A trend was found for higher ongoing pregnancy rates with highly purified hMG vs recombinant FSH (rFSH) (27% vs 22%) [3].

A subsequent meta-analysis evaluating only gonadotropin releasing hormone (GnRH) agonist protocols involving 2,519 IVF cycles found a significantly higher live birth rate with hMG (25.5%) vs rFSH (21.6%) [4].

A meta-analysis by Al-Inany et al included both long GnRH agonists and short GnRH antagonist protocols and still found higher pregnancy rates with hMG [5]. There are less data concerning the benefits of having LH in the controlled ovarian hyperstimulation (COH) protocol when one exclusively uses a GnRH antagonist protocol. Although meta-analyses always favor prospective studies, and there is always the possibility of potential selection biases when performing a retrospective study, there are still meaningful conclusions that can be reached from a large retrospective study. This is especially true if one is evaluating a group not likely to ever be studied in a prospective study.

The objective of this study was to evaluate whether adding LH to FSH stimulation increases the pregnancy
outcome following IVF-ET in women with diminished oocyte reserve using a GnRH antagonist protocol. Furthermore, similar comparisons of women using all FSH vs LH added would be retrospectively compared in a large group of women with normal oocyte reserve.

Materials and Methods

A retrospective review of all IVF cycles using GnRH antagonists were used from January 2, 2003 through April 30, 2010. To increase the statistical power, all cycles were included so that a woman not conceiving previously could have been used multiple times. Though some would claim that using the same couple more than once could introduce a bias, on the other hand, studies only using a couple’s first IVF cycle may select a population with a better prognosis. By including all cycles, this study would include women with a worse prognosis since they could have failed to conceive despite several previous cycles.

The data were stratified not only according to poor responders (poor responder arbitrarily assigned to a group having four or less oocytes retrieved) vs normal responders, but according to two age groups (≤ 39 and 40-42 years). There were no restrictions for day 3 serum FSH and estradiol (E2) levels. Cycles having intracytoplasmic sperm injection were included.

Comparisons were made using Chi-square analysis and included clinical pregnancy rate/transfer (ultrasound evidence of pregnancy at eight weeks) viable pregnancy rate (live fetus at 12 weeks), and live delivered pregnancy rates and implantation rate.

The relative amount of LH to FSH could have been as little as 25% and as much as 50%. The antagonists were either cetrotrelix or ganirelix and were used at a dosage of 250 mcg, once at least one follicle reached an average diameter of 14 mm. If day 3 serum FSH was > 12 mIU/ml or serum E2 > 50 pg/ml, or a diminished antral follicle count was found on day 3, there was a marked reduction in the dosage of gonadotropins [6, 7].

Results

The comparison of pregnancy outcome according to taking all FSH vs FSH plus LH in normal responders using a GnRH antagonist is shown in Table 1.

The comparison of pregnancy outcome according to taking all FSH vs FSH plus LH in poor responders using a GnRH antagonist is shown in Table 2.

Combining all data (all ages and good and poor responders), there was a significantly higher clinical pregnancy rate in those taking FSH plus LH (423/1,066, 39.7%) vs those taking all FSH (248/743, 33.4%) with p = 0.007, chi-square analysis.

Significantly higher viable and live delivered pregnancy rates were also seen in the FSH plus LH group (35.5% and 32.3% vs 28.3% and 25.8%) with p = 0.007 and p = 0.0038, respectively.

If one evaluates separately the poor responder group (Table 2), no significant differences were found or even a trend for superiority of COH regimens adding LH to the FSH. For poor responders, the pregnancy rates for those taking all FSH vs LH added to FSH were: clinical pregnancy rate/transfer – 25.5% (103/404) vs 26.4% (60/227), viable pregnancy rate/transfer – 20.5% vs 22.5%, live delivered pregnancy rate/transfer – 17.8% vs 20.3% (Chi-square analysis = no significance). Evaluating the percentage of first IVF cycles in each group, there were 350/814 (43.0%) first cycles in normal responders taking FSH and LH vs 150/337 (44.5%) of those women using FSH exclusively.

Discussion

A recent Cochrane meta-analysis evaluated the effect of recombinant LH for COH in 14 prospective trials involving 2,612 women. However 11 of the 14 trials involved 2,396 women using a GnRH agonist and thus only three trials involving 216 women used a GnRH antagonist [8].

Only seven of the 13 trials provided ongoing pregnancy rates (only three provided live births) and this Cochrane analysis did not find that the addition of LH improved the outcome. A priori, because of prolonged pituitary suppression, one might suspect that adding LH would be even more important for GnRH agonist cycles which rep-
resented the majority of patients in this study [8]. Interestingly three trials in this Cochrane meta-analysis used only poor responders and this sub-group did show that adding LH improved pregnancy outcome [8-11]. Though retrospective, the present study included 1,810 women which thus had five times the power of the three prospective studies evaluating GnRH agonist protocols. Only two of the 14 prospective studies in the recent Cochrane meta-analysis evaluated the live birth rates and these two studies used GnRH agonists not antagonists. The data in this study provided the live birth rates.

Theoretically women taking GnRH agonists should have a greater need for addition of LH to FSH stimulation, compared to GnRH antagonists because of prolonged pituitary suppression and longer recovery to producing LH when using the former. Yet this retrospective data showed a significant 20% increase in live delivered pregnancy rates when adding LH to FSH from the early follicular phase using a GnRH antagonist protocol for COH.

Retrospective studies have several inherent flaws, so one has to favor conclusions made from well-designed prospective studies. However, frequently prospective studies are flawed by type I statistical errors, i.e., insufficient power. Theoretically, combining several prospective studies and evaluating these combined data as in a meta-analysis can obviate type I errors; however until there are sufficient numbers of prospective studies evaluating addition of LH with GnRH antagonist protocols, the data from larger retrospective studies in deciding COH protocols may be considered.

Using more than one treatment cycle per patient in a retrospective study adds a potential bias that if one group has more first cycles, that group could be favored. However, there was no favoring of first cycles for FSH only vs LH plus FSH and the data from the largest of the groups was provided under results. The authors purposely included all cycles not only to increase the power but to include more difficult patients, i.e., those failing to conceive in previous IVF-ET cycles. One of the problems in prospective studies is that they tend to exclude more difficult cases and favor the ones with the best prognosis.

It is interesting that with the recent meta-analysis by Mochtar et al., the only subgroup that improved with LH and FSH were the poor responders [8]. In contrast in this retrospective study, this group did not show an advantage of adding LH to FSH. There was the same 20% increase in live delivery pregnancy rates in poor responder women ≤ 39.9 years adding LH, so the lack of significance could be from a type I error. However it should be noted that in women 40-42 years of age, there was a 20% decreased live delivered pregnancy rate in those taking LH and FSH.

References


The effect of diminished oocyte reserve in younger women (age ≤ 37) on pregnancy rates in natural cycles

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Summary

Purpose: To determine the relative confounding effect of diminished oocyte reserve on the chance of successful pregnancy in non-in vitro fertilization-embryo transfer (IVF-ET) cycles. Materials and Methods: Matched controlled study comparing pregnancy outcome in women aged ≤ 37 years with severely decreased oocyte reserve as manifested by a day 3 serum follicle stimulating hormone (FSH) ≥ 15 mIU/ml compared to women with normal oocyte reserve (serum FSH ≤ 8 mIU/ml). Couples were excluded if they did not have tubal patency or a semen analysis that required IVF-ET. Only couples that tried at least three natural cycles (unless pregnancy occurred first) were included. Results: The live delivered pregnancy rates within a maximum of five cycles of luteal phase support with progesterone (P) or at most mild FSH stimulation, or intrauterine insemination for mild male factor or cervical factor was 33.3% (8/24) with increased day 3 FSH and 62.5% (16/24) for the normal group (p = 0.08, Fisher’s exact test). Conclusion: Women with marked oocyte depletion are half as likely to conceive with assisted reproductive techniques compared to women with normal oocyte reserve.

Key words: oocyte depletion are half as likely to conceive with assisted reproductive techniques compared to women with normal oocyte reserve.

Introduction

One study from a world renowned in vitro fertilization (IVF) center concluded that live deliveries simply did not occur following the transfer of embryos that appeared to have normal morphology if the serum day 3 follicle stimulating hormone (FSH) ever exceeded 15 mIU/ml [1]. Based on this aforementioned study and others, the philosophy used by many specialists in reproductive endocrinology and infertility is to advise women whose day 3 serum FSH is > 15 mIU/ml that pregnancy with their own oocytes is highly unlikely and that they should proceed to using donor oocytes [1].

In contrast, it has been demonstrated that a reasonably good live-delivered pregnancy rate can be achieved following in vitro fertilization-embryo transfer (IVF-ET) in women who not only had serum FSH levels > 15 mIU/ml but whose response following gonadotropin stimulation was so poor that they only had a single embryo to transfer [2]. The explanation for these opposite conclusions is that women with diminished oocyte reserve are extremely sensitive to the adverse effects of high-dose FSH controlled ovarian hyperstimulation and that mild stimulation or even natural cycles are needed to attain good pregnancy rates combined with luteal phase progesterone (P) support [3]. In fact, live deliveries have been recorded by reversing apparent menopause by FSH receptor modulation without IVF-ET in women whose serum FSH exceeded 100 mIU/ml [4-7].

The possibility exists that the use of even mild gonadotropin stimulation during IVF-ET in this group of women with diminished oocyte reserve may have negative consequences in some women in this group, though not nearly as high a percentage following high dosage FSH stimulation. The objective of this present study was to compare pregnancy rates in women with normal oocyte reserve vs women with marked diminished oocyte reserve with serum FSH levels exceeding 15 mIU/ml without IVF-ET where purposeful FSH stimulation was not used to create multiple follicles but where FSH would be only used, if at all, in extremely low dosages to boost one follicle to maturity.

Materials and Methods

A matched controlled study was performed to compare pregnancy rates in women whose FSH was increased above 15 mIU/ml to those with normal oocyte reserve. Because of tubal patency and relatively normal semen parameters, IVF-ET was not considered needed. All women aged ≤ 37 years with a day 3 serum FSH > 15 mIU/ml who had bilateral patent fallopian tubes and a male partner with a normal semen analysis, resulting in a normal post-coital test, were enlisted in the study. They were matched to the very next woman meeting these same criteria whose age was within two years of the woman to whom they were being matched (as long as the age was ≤ 37) with normal oocyte reserve as manifested by a day 3 serum estradiol (E2) < 50 pg/ml and a serum FSH ≤ 8 mIU/ml.

All women received vaginal P in the luteal phase. They were exclusively treated with P if they achieved a follicle in a natural cycle of 18-24 mm with a serum E2 > 200 pg/ml [8, 9]. If they did not achieve a mature follicle they were given a small boost of low dosage (75 IU usually, occasionally 150 IU) of gonadotropins [8, 9].

The principle used for those with increased FSH was not to start any exogenous FSH until the rise of endogenous E2 decreased the serum FSH level below 11 mIU/ml [10]. Occasionally ethinyl E2 at usually 20 micrograms per day (rarely increased to 40 micrograms/day) was used early in the follicu-
lar phase to lower serum FSH without adding to the serum E2 level to restore down-regulated FSH receptors in women not showing follicular development with increased FSH [11]. The principal was never use any exogenous FSH if the serum FSH was > 11 mIU/ml [10]. Ethinyl E2 was also used to lengthen the short follicular phase or prevent premature luteinization [12].

The P dosage was titrated to achieve a mid-luteal homogeneous hyperechogenic endometrial echo pattern [13, 14]. Intrauterine insemination was not employed but only natural intercourse. The women selected had to have primary or secondary infertility of over one year duration. Pregnancy rates after a maximum of five natural cycles were determined and compared – a minimum of three treatment cycles for both partners was required.

Results

The clinical pregnancy rate for a maximum of five treatment cycles was for high FSH – 10/24 – 41.6% vs for normal FSH – 17/24 – 70.8% (chi-square analysis p = 0.08).

The live delivered pregnancy rate was for high FSH – 8/24 – 33.3% vs for normal FSH – 15/24 – 62.5% (Fisher’s exact test p = 0.08).

The average number of cycles for conception in the high FSH group was 3.1 vs 3.2 for the normal group.

The mean FSH for the normal oocyte reserve group was 6.1 ± 2.8 mIU/ml vs 20.6 ± 5.5 mIU/ml for the diminished oocyte group.

Discussion

Live-delivered pregnancies are half as likely to occur following proper correction of ovulatory defects, e.g., correcting short follicular and luteal phases in infertile women with marked diminished oocyte reserve vs infertile women even with natural intercourse. Certainly the majority of women with serum FSH levels > 15 mIU/ml would prefer a 33.3% live pregnancy rate within five cycles of intercourse as opposed to going into a donor oocyte program.

We purposely chose a group with more severe depletion of oocyte reserve, i.e., those with serum FSH was > 15 mIU/ml. More women with diminished reserve may have day 3 serum FSH levels from 12 - 14 mIU/ml. This group from our anecdotal experience has even higher pregnancy rates than this study group. Yet even with this less severely oocyte depleted group, a frequent recommendation is to go immediately into the donor oocyte program.

References

Younger women with diminished oocyte reserve are not more prone to meiosis errors leading to spontaneous abortion than their age peers with normal oocyte reserve

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Introduction

From the early days of in vitro fertilization-embryo transfer (IVF-ET), most IVF centers reported very poor pregnancy results following the transfer of embryos from women with diminished oocyte reserve even if they were younger [1-6]. Based on the demonstration that chromosomal analysis of 59 aborted fetuses, 19 of 33 (57%) with abnormal karyotypes had elevated day 3 serum follicle-stimulating hormone (FSH) levels or elevated serum estradiol levels which were significantly higher than the 7 of 26 (27%) who had normal karyotypes. One study concluded that younger women with diminished oocyte reserve are more prone to meiosis errors leading to the oocyte quality of a woman of advanced reproductive age rather than her age peers [7]. Although in the modern IVF era, some of the top IVF-ET centers still report extremely low pregnancy rates despite marked improvement in IVF technology; many reach the conclusion that younger women with diminished oocyte reserve have not just poor quantity of oocytes but poor quality akin to women of advanced reproductive age who are known to be prone to meiosis errors. Thus they are likely to produce embryos with aneuploidy that either do not implant or spontaneously abort [8, 9].

However, other studies do not agree with these studies finding very low live delivery rates following IVF-ET in women with diminished oocyte reserve [10-12]. These IVF centers finding normal pregnancy rates in women with diminished oocyte reserve suggest that the reason for poor pregnancy results found by the other aforementioned studies was the inappropriate use of high FSH stimulation protocols [13].

The most likely cause of any given spontaneous abortion would be related to chromosome abnormalities in all women irrespective of oocyte reserve, especially in women supplemented with progesterone from early luteal phase throughout the first trimester as in women undergoing IVF-ET. The present study evaluated rates of spontaneous abortion according to age and according to degree of diminished oocyte reserve in women undergoing IVF-ET in which women with day 3 serum FSH > 11 mIU/ml were stimulated with mild FSH protocols.

Both authors reasoned that if no increase in spontaneous abortion rate was found according to those with or without diminished oocyte reserve, this finding would abrogate the widely held concept that oocytes from women with diminished oocyte reserve associated with high day 3 serum FSH levels are intrinsically more prone to meiosis errors and thus are highly unlikely to produce live pregnancies [9].

Materials and Methods

The first pregnancies achieved by women undergoing IVF-ET during a ten-year period at one university based IVF center were stratified into four groups based on the age of the female partner: ≤ 35, 36-39, 40-42, and 43-44 years. Each of the four...
groups were further subdivided into four groups based on their day 3 serum FSH level (mIU/ml) during their screening cycle: ≤ 11, 12-14, 15-17, and > 17 mIU/ml.

The clinical pregnancy rates as determined by a gestational sac at six weeks were determined for each of these subgroups. The spontaneous abortion rate were equal to the percentage of women with a gestational sac who did not deliver at least one live baby.

All women with a day 3 serum FSH of ≥ 12 mIU/ml were treated with mild ovarian stimulation ranging from 150 units of FSH from days 3-5 or a completely natural cycle according to antral follicle count and level of day 3 serum FSH as previously described [12, 13]. Most cycles used gonadotropin releasing hormone (GnRH) antagonists.

The majority of IVF-ET cycles in women with normal day 3 serum FSH level used traditional controlled ovarian hyperstimulation commencing at 225 or 300 IU of FSH. They more often used GnRH agonists leuprolide acetate from the mid-luteal phase. All women were treated with varying vaginal progesterone from the day after oocyte retrieval throughout the first trimester.

Only IVF cycles transferring on day 3 were included in the study to allow uniformity. Also, to prevent wrongly placing a woman with diminished oocyte reserve into the normal FSH category, women with elevated day 3 serum estradiol > 50 pg/ml but whose serum FSH was ≤ 11 mIU/ml, were not included in the study.

Statistical comparisons between groups were performed by either chi-square analysis or Fisher’s exact test.

**Results**

The clinical (ultrasound evidence of pregnancy at six weeks), viable (live fetus at 12 weeks), live delivered pregnancy rates, and spontaneous abortion rates in women aged ≤ 39 are seen in Table 1. For women aged ≤ 35 or 36-39 years, there were no significant differences in spontaneous abortion rates between the four FSH groups or even a trend for higher spontaneous abortion rates with increasing day 3 serum FSH (p = NS, Fisher’s exact test).

For women aged ≤ 35 years with normal day 3 serum FSH, the spontaneous abortion rate was 13.6% (97/717). For women aged 36-39 years with normal ovarian reserve, the spontaneous abortion rate was 21.1% (78/369). The spontaneous abortion rate was significantly higher in women aged 36-39 compared to those aged ≤ 35 years in women with normal oocyte reserve (p = 0.0017, chi-square analysis). Table 2 provides the same data for women aged 40-44 years. For women aged 40-42 years there was no significantly higher spontaneous

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<td>369 44 14 35</td>
</tr>
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<td>% clinical pregnancy/transfer</td>
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<table>
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<td>26 3 0 2</td>
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<td>No. miscarriages/clinical pregnancies</td>
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abortion rate in those women with serum FSH ≥ 12 mIU/ml (41.6%, 27/65) compared to women with normal FSH of ≤ 11 mIU/ml (31.8%, 56/176) (p = 0.20, chi-square analysis). There were no differences in spontaneous abortion rates in women aged 40–42 years with mildly diminished oocyte reserve (FSH 12–14 mIU/ml) vs severely depleted (FSH > 17) - 45.2% (14/31) vs 39.1% (9/23) (p = 0.78, Fisher’s exact test).

For women aged 43–44 years, the spontaneous abortion rate was significantly higher for those women with increased day 3 serum FSH levels (84.2%, 16/19) vs those with normal serum FSH (40.6%, 13/32) with p = 0.006, chi-square analysis.

Considering women with normal FSH, there was a significantly higher spontaneous abortion rate comparing the four age groups, i.e., 13.5% (97/717) for women aged ≤ 35 vs 21.1% (78/369) for women aged 36–39 (p = 0.005, chi-square analysis) vs 31.8% (56/176) for women aged 40–42 (p = 0.009, chi-square analysis) vs 40.6% (13/32) for women aged 43–44 (p = 0.44, chi-square analysis).

Fetal karyotypes were available in 217 of the 320 products of conception. A female karyotype was seen in 124 of the 217 products of conception and a male karyotype in 93 abortuses. A chromosomal abnormality, i.e., trisomy, monosomy, triploidy, or tetraploidy was found in 71 of the 124 female fetuses (57.7%) vs 68 of 93 (73.1%) of male fetuses. The difference in male vs female could be related to possible maternal contamination. Looking just at male fetuses with abnormal karyotype, 40 of the 68 aborters (58.8%) were trisomies, 16 were monosomies (23.5%), ten were triploidies (14.7%), and two were tetraploidies (2.9%). Forty-one of the 61 (67.2%) women with normal oocyte reserve who had spontaneous abortions with male products of conception had chromosomal abnormalities vs 27 of 32 (81.7%) of those with diminished oocyte reserve (p = 0.13, chi-square analysis).

**Discussion**

Considering that chromosomal abnormalities are considered the most common cause of any given spontaneous abortion, it is a reasonable assumption that the cause of the majority of spontaneous abortions in these heavily progesterone supplemented women was fetal aneuploidy [14]. It is well-known that advanced maternal age is one of the most important associations with risk of aneuploidy. The present study certainly confirms this by showing a significantly higher spontaneous abortion rate with advancing age of the four age groups studied even in women with normal oocyte reserve.

However for the two younger groups (aged ≤ 39 years), there was no difference in spontaneous abortion rates within an age group according to the degree of oocyte reserve. There was not even a trend for higher spontaneous abortion rates in the group with the least ovarian reserve as manifested by a day 3 serum FSH of > 17 mIU/ml vs the normal reserve group with FSH ≤ 11 mIU/ml.

It was not until age 40–42 years that a significant increased rate of spontaneous abortion was found in the group with diminished vs normal ovarian reserve. However, even in this group, the differences were not great with a rate of 39.1% in the FSH group > 17 mIU/ml vs 31.6% for the FSH group ≤ 11 mIU/ml. The largest difference was found in the oldest group of 43–44-year-olds. Interestingly, the spontaneous abortion rate in the group of women aged 43–44 years with normal FSH was very similar to the group of 40–42-year-olds with diminished oocyte reserve.

These data are consistent with the concept that the oocytes with the least risk of meiosis errors are favored for selection of antral follicles from which the dominant follicle develops. These data are consistent with the concept that the etiology for younger women having diminished ovarian reserve is not from an acceleration of oocyte atresia in the natural order of the best ones first. Instead, these data are consistent with the concept that the main etiologic factor causing diminished oocytes reserve is a destructive process damaging significant portions of the ovaries; however what portion has been spared has the same proportion of oocytes with less risk of meiosis errors as their age peers, but only have a quantity remaining more akin to women of advanced reproductive age [15].

Tables 1 and 2 for women aged ≤ 42 years show no difference in live-delivered pregnancy rates in those with diminished vs normal oocyte reserve. These data are consistent with the concept that it is not the quality of the oocyte but the use of high FSH dosage of controlled ovarian hyperstimulation (COH) regimens that is responsible for the very low pregnancy rates reported by some of the most successful IVF centers for women with diminished oocyte reserve [12, 13]. One hypothesized mechanism of how high-dose stimulation protocols are responsible for very low pregnancy rates in women with diminished oocyte reserve is that the FSH receptors, which are very susceptible to down regulation, may be suppressed by a further rise in the serum FSH and some FSH dependent implantation factor is not produced leading to an embryo that looks normal but does not implant [12, 13, 15].

However, another possibility is somehow the high-dose FSH stimulation makes oocytes from women with diminished oocyte reserve to become more prone to meiosis errors, but in the presence of endogenous or mild exogenous FSH stimulation, these oocytes are not any more prone to meiosis errors than their age peers.

There is one inherent bias in these statistics which probably affects the pregnancy rate more than the spontaneous abortion rate. Our IVF center is known to take highly difficult cases with a poor prognosis. The majority of the women with elevated day 3 FSH have been refused IVF by other IVF centers because of their elevated day 3 FSH level. Thus frequently they are having their first IVF cycle with our group or possibly the second. In contrast, the normal FSH group has frequently failed after many cycles elsewhere or even in our own
practice either for unknown reasons or poor endometrial thickness, etc. Thus if this study was to determine the likelihood of live delivered pregnancies according to day 3 FSH levels, we would have only compared first IVF cycles. However for a miscarriage study we chose first pregnancies. The ideal study would use mild ovarian stimulation in all groups to exclude the confounding effect of hyperstimulation on pregnancy rates.

These data support the contention that of at least two possible mechanisms for diminished oocyte reserve, i.e., a destructive process damaging a large percentage of ovarian tissue, thus resulting in low number of remaining follicles, but the remainder of the ovarian tissue has the same percentage of normal oocytes as their age peers (just less of them). Alternatively another possibility is that there is a more rapid rate of atresia in the normal fashion, leaving not only less quantity of oocytes, but less quality ones more prone to meiosis errors. The former mechanism seems to be the more prevalent mechanism in women aged ≤ 39 years. When a woman reaches age 40-42 years based on these data, the percentage of women with a somewhat more rapid rate of atresia, as the mechanism of oocyte depletion, increases enough to cause a significant increase in spontaneous abortion rate. However, the spontaneous abortion rate is not that much higher thus suggesting that a good number of them have the destructive mechanism at their etiology. In contrast, in women aged 43-44 years, the majority with depleted oocyte reserve are just part of the bell-shaped curve and have defective oocytes. Nevertheless, live deliveries are still possible in a minority of the cases going through IVF.

There is still one more hypothesis that can explain these data. Aging of the mitochondria may explain the increased risk of aneuploidy with advancing age rather than advancing rise in day 3 FSH, or continued depletion of antral follicles, inhibit B, and the anti-Müllerian hormone. This theory contends that most oocytes are prone to meiosis errors but there may be one or at best a few with the best mitochondria that can allow proper chromosome segregation. However advancing age causes aging of mitochondria in all cells including the one or two oocytes in the cohort that have the “best” mitochondria, thus leading to errors of non-disjunction even in these “best” follicles. Indeed there are data suggesting that there are only an average of 1.8 normal embryos in a given group of embryos produced by an IVF-ET cycle, even in women with more embryos created by a high-dose FSH protocol vs women with less embryos produced by mild FSH stimulation [16].

References
[16] Baart E.B., Martini E., Eijkemans M.J., Van Opstal D., Beckers B.H., Slovis, J.H. Check. Address reprint requests to: J.H. CHECK, M.D., Ph.D. 7447 Old York Road Melrose Park, PA 19027 (USA) e-mail: laurie@ccivf.com
Intrauterine insemination (IUI) does not improve pregnancy rates in infertile couples where semen parameters are normal and postcoital tests are adequate

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Summary

Purpose: To determine if intrauterine insemination (IUI) improves pregnancy rates in couples with a correctable ovulatory defect but a male partner with an apparent normal semen analysis and a normal postcoital test. Materials and Methods: A prospective evaluation of clinical live delivered pregnancy rates following the first cycle where follicular maturation was demonstrated naturally or with a follicle maturing drug. The couples were given the option of IUI. Results: The live delivered pregnancy rates per IUI cycle were similar with intercourse only vs addition of IUI (18.7% vs 21.4%). Conclusions: There is no evidence to support the notion that IUI improves pregnancy rates in circumstances where the semen analysis and postcoital tests are normal.

Key words: Intrauterine insemination; Postcoital test; Normal semen analysis.

Introduction

A woman’s most fertile cervical mucus immediately precedes her peak serum estradiol (E2). Peak serum E2 induces a luteinizing hormone (LH) surge which causes the extrusion of the oocyte from the follicle approximately 36-40 hours later. The hormonal consequences of the rise in serum LH lead to subsequent changes in hormonal secretion by the granulosa-theca cells leading to a drop in the serum E2 and an increase in serum progesterone (P).

The rise in P and decrease in E2 leads to diminishing mucus quality so that right at the time of ovulation the mucus is inferior to 36-40 hours before and may not allow the sperm to progress through the cervical mucus to reach the uterine cavity [1]. Thus one theoretical advantage of intrauterine insemination (IUI) where processed sperm is placed in the uterine cavity is that it allows more immediate contact of fresh sperm with the oocyte rather than sperm that is 40+ hours old where there may be failure to maintain the sperm’s fertilization potential [2].

The present study aimed to determine if the addition of IUI despite a normal post-coital test in couples with no apparent male factor issues may improve the pregnancy rate.

Materials and Methods

Couples with a minimum of one year of infertility with male partner’s normal semen analyses and female partner’s with correctable ovulation disorders were recruited. Couples were asked in the beginning of the cycle whether they would proceed with IUI even if the postcoital test was sufficient or not. They were advised that a theoretical advantage of the IUI was to deposit sperm closer in time to ovulation since cervical mucus may be impenetrable at this time. The IUI was performed about 40 hours after either a spontaneous LH surge or the injection of 10,000 units human chorionic gonadotropin (hCG). Ultrasound demonstrated release of the oocyte as evidenced by shrinkage of the follicle by at least five mm.

Comparisons were made of pregnancy rates in the first cycle to those adding IUI or not. If the postcoital test failed to demonstrate any sperm with progressive motion, they would be eliminated from the study. Postcoital tests were performed at the time of follicular maturity (at least one follicle of 18-mm average diameter with a serum estradiol > 200 pg/ml and prior to the LH surge).

Sperm concentration, percent motility, morphology using strict criteria, antisperm antibodies and hypo-osmotic swelling tests were also performed on each initial sperm specimen.

Results

Three couples were eliminated for an insufficient post-coital test leaving 72 study couples. There were 16 couples who chose to not have an IUI (22.2%) and 56 who chose to add IUI (77.8%).

Pregnancies were achieved in 16/56 couples with intercourse (28.6%) vs 15/56 (26.7%) of those adding IUI. Live deliveries occurred in 3/16 (18.7%) with intercourse only vs 12 (21.4%) with IUI added (Fisher’s exact test showed no significant difference in pregnancy rates).

Discussion

Griffith and Grimes performed a meta-analysis and concluded that the postcoital test was not very good at predicting fertility potential [3]. We do not agree with their conclusions since Griffith and Grimes did not select studies where the postcoital test was determined accord-
ing to the proper blood tests confirming the right timing [1, 4]. Interestingly, Griffith and Grimes calculated that based on their conclusion the postcoital test is not valid in that 50 million dollars per year of fertility testing may be wasted on this test. Some modern OB/GYN textbooks refer to the postcoital test as an archaic test that used to be performed but today has no value.

Many fertility centers instead of doing postcoital tests simply perform IUI each cycle empirically. The charge for IUI varies from $250.00 to $2,000.00 and some centers perform them two times in a cycle. The charge of a one time postcoital test is $75.00 and the average charge of IUI is about $600.00. Assuming three IUIs per pregnancy, and since this study found no benefit to the IUI if the postcoital test was normal, it can be stated that the policy of circumventing the postcoital test and simply performing an IUI wastes 1.2 billion dollars per year if the calculation of Griffith and Grimes is used (24 x 50 million).

These data suggest that if lack of longevity of fertilization potential by some males’ sperm can be a cause of infertility, it is not frequent enough to allow improved pregnancy rates with IUI. Thus empirical use of IUI for unexplained infertility is not cost effective. This policy of using empirical IUI for unexplained infertility dates back to 1991 with a publication finding a reasonably good pregnancy rate using superovulation and IUI instead of in vitro fertilization for unexplained infertility [5]. However, it is not clear what part of the treatment was responsible for the pregnancy – the follicle maturing drugs, the hCG injection, the IUI or the luteal phase support with P [5].

References


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Low hypo-osmotic swelling tests correlate with low percent motility and age of the male

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Summary

Purpose: To determine if sperm motility and hypo-osmotic swelling (HOS) test scores are associated. Furthermore the study would determine if the chance of having a subnormal HOS test increases as motility levels decrease. Finally the study would determine if age, motility, and HOS test scores are independent factors or if they interact. Materials and Methods: A retrospective analysis of males of all ages with a normal sperm concentration of > 20 x 10⁶/ml is presented. Males were evaluated separately according to age (< 40 vs ≥ 40 years) for percent progressive motility and HOS test scores. The percent motility was assessed according to percentage in deciles. Results: A significantly higher percentage of males with low motility have low (< 50%) HOS test scores irrespective of age. The odds ratio of males < 40 years having an abnormal HOS test score is 6.73 times higher and is 8.23 times higher for males ≥ 40 years. As age increases, there is a significantly larger proportion of males with abnormal HOS test scores (6% to 13%). Conclusions: Factors that cause an abnormality in the functional integrity of the sperm membrane, as evidenced by a low HOS test score, can also have a negative effect on motility.

Key words: Hypo-osmotic swelling test; Sperm motility; Age.

Introduction

Low motility is a well-known factor associated with male subfertility [1]. A low hypo-osmotic swelling (HOS) test score (< 50%) is a less known factor associated with male subfertility [2]. However, when the HOS test is abnormal, it predicts male subfertility better than motile density or morphology [3].

The HOS test abnormality is interesting because it is unique among male factor issues in that a low level allows normal fertilization but impairs implantation of the embryos [4]. The objective of the study was to determine if there is an association with strictly low motility in the presence of adequate sperm concentration with subnormal HOS test scores.

There were three questions hoped to be answered by this study:

Question 1 – Are sperm motility and HOS scores associated?

Question 2 – Does the chance of having a subnormal HOS test increase as the motility levels decrease?

Question 3 – Are age, motility, and HOS independent factors or do they interact?

Materials and Methods

Only males with a sperm concentration of ≥ 20 x 10⁶/ml were chosen. Males with normal (≥ 50%) or subnormal (< 50%) motility percentages were determined.

Further subdivided by age of the male (< 40 vs ≥ 40 years). Percentage of low HOS scores were determined according to 10% deciles in the low motility group.

The correlation of abnormal HOS scores and low motility is shown in Table 1. Only 2.9% (78/2659) of males < 40 years with normal motility had abnormal HOS test scores vs 16.9% (128/757) of men with low percentage motility (< p < 0.001, chi-square analysis). Similarly a significantly larger proportion of males ≥ 40 years had abnormal HOS test scores when the motility was < 50% (31.6%) vs when the motility was ≥ 50 (5.3%).

Question 1 – Are sperm motility and HOS scores associated?

The correlation of abnormal HOS scores and low motility is shown in Table 1. Only 2.9% (78/2659) of males < 40 years with normal motility had abnormal HOS test scores vs 16.9% (128/757) of men with low percentage motility (< 0.001, chi-square analysis). Similarly a significantly larger proportion of males ≥ 40 years had abnormal HOS test scores when the motility was < 50% (31.6%) vs when the motility was ≥ 50 (5.3%).

The odds ratio in men < 40 years for having abnormal HOS in the presence vs absence of low motility was 6.73 (95% confidence interval, 5.01, 9.04 (p < 0.001). Thus the odds of a male < 40 years having an abnormal HOS test score were 6.73 times higher for males with low motility than for males with normal motility. For men ≥ 40 years, the odds ratio was 8.23 (95% confidence interval 6.03, 11.23, p < 0.001).

Question 2 – Does the chance of having a subnormal HOS test increase as the motility percentage decreases?

Table 2 lists the number and percentage of abnormal HOS tests in males with diminished motility according to deciles of motility. Table 2 indicates that the rates of abnormal HOS tests increases as the motility rates
decrease in those males with abnormal motility in both age groups \((p < 0.0001, \text{chi-square analysis})\).

**Question 3** – Are age, motility, and HOS independent factors or do they interact?

To answer this question, one can consider a saturated log linear model with three factors: age \((< 40, \geq 40)\), motility status \((40 - 49\%, 30 - 39\%, 20 - 29\%, < 20\%)\), and HOS \((\text{normal, abnormal})\). A saturated model allows for the testing of interaction between the factors. In this model, there was no significant three-way interaction between HOS scores, motility, and age. There was a significant two-way interaction between HOS and age. There was a significant two-way interaction between HOS and low motility for both age groups. As age increases, the HOS abnormality increases significantly from 6% to 13%.

### Discussion

There is evidence that the HOS abnormality may be caused by the presence of a toxic protein, as evidenced by correction of the HOS abnormality by the protein digestive enzyme chymotrypsin \([5-7]\). Not only does the chymotrypsin improve the HOS score, but also allows improvement of pregnancy rates approaching normal \([5-7]\).

Based on these data, it would appear that this toxic protein adversely affects the motility in some but not in all males with low HOS test scores. These data confirm previous studies suggesting that the HOS abnormality increases with advancing age of the male \([8]\).

Thus these data suggest that factors that can cause a functional impairment of the sperm membrane leading to low HOS test scores may also adversely affect motility.

### References


Effect of triple line vs isoechogenic endometrial texture on pregnancy outcome following embryo transfer according to use of controlled ovarian stimulation (COH) or estrogen/progesterone replacement

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Division of Reproductive Endocrinology and Infertility, Camden, NJ
²Philadelphia College of Osteopathic Medicine, Department of Obstetrics and Gynecology, Philadelphia, PA (USA)

Summary

Purpose: To determine if pregnancy rates following embryo transfer are reduced if the endometrial echo pattern in the late proliferative phase is isoechogenic (IE) vs triple line (TL). Methods: Pregnancy and implantation rates were compared according to TL vs IE pattern in the late proliferative phase in women having in vitro fertilization-embryo transfer (IVF-ET), frozen ET, and transfer of embryos derived from donor oocytes. Results: There was no difference in pregnancy rates with IE vs TL pattern with fresh or frozen ET or in donor egg recipients. The degree of ovarian reserve did not affect the pregnancy rates according to endometrial echo pattern. Conclusions: The presence of an IE pattern in the late proliferative phase should not influence the treating physician to either cancel the cycle and withhold human chorionic gonadotropin (hCG) injection or freeze all embryos and defer transfer.

Key words: Endometrial echo pattern; Late proliferative phase; Embryo transfer; Diminished oocyte reserve.

Introduction

Endometrial echo patterns are determined by the comparison of the echogenicity of the endometrium to that of the myometrium and the distinction of a central echogenic line within the endometrium. Smith et al. [1] were the first to describe these patterns during in vitro fertilization-embryo transfer (IVF-ET) cycles: 1) Triple Line (TL) – hypo-echogenic with well-defined outer walls and central echogenic line; 2) Isoechogenic (IE) – isoechogenic with a poorly defined central echogenic line; 3) Homogeneous hyperechogenic (HH) – hyperechogenic with no visualization of a central line.

Gonen et al. in 1990 concluded that for IVF-ET using a clomiphene citrate-human menopausal gonadotropin regimen the only echo pattern that correlated with good pregnancy rates when evaluated at the peri-ovulatory time was the TL pattern [2]. However, Check et al. in 1991 did not find the TL pattern to be superior to the IE pattern when using a luteal phase leuprolide acetate-gonadotropin regimen [3].

The original study by Check et al. evaluated 85 IVF-ET cycles. A subsequent larger retrospective study performed during the “old era” of IVF-ET of 273 IVF-ET cycles using the luteal phase leuprolide acetate-gonadotropin regimen found a clinical pregnancy rate of 24.5% (37/151) with TL, 17.0% (17/100) with IE and 0% (0/22) for HH pattern [4]. This aforementioned study showed a significantly higher pregnancy rate for TL or IE vs HH but did not have enough power to show a significant difference between TL and IE [4].

The adverse effect of an HH pattern in the late proliferative phase was subsequently confirmed by Noyes et al. in 1996 in women undergoing IVF-ET who had in utero exposure to diethylstilbestrol (DES) [5]. Noyes et al. actually found that exposure to DES increased the frequency of the HH pattern from 15.0% to 40.5% [5]. Check et al. found the HH pattern to be present in 8% of cycles [4]. Noyes et al. did not find any clinical pregnancies in 18 DES exposed women with the HH pattern but interestingly did not see any adverse effect of the HH pattern in non-DES exposed women. However with DES exposed women they had a 60% clinical pregnancy rate (PR) per transfer with TL but only a 22.2% PR with IE [5].

Based on our previous data we have a policy to defer fresh ET and cryopreserve all embryos if an HH pattern is seen on the day of hCG injection [3,4]. However, a TL or IE pattern does not influence the decision of whether to transfer the embryos or cryopreserve. The aim of the present study was based on the non-significant trends for lower PRs with IE pattern in the “old” days of IVF-ET, to re-evaluate this issue with a study with much more power in the modern era of IVF. With improvement in IVF technology, and thus pregnancy rates, if there was a trend for superior pregnancy rates with the TL pattern it might no longer be present.

Furthermore the study would compare the influence of TL vs IE pattern in different clinical circumstances: 1) IVF-ET with normal oocyte reserve vs diminished oocyte reserve; 2) Frozen ET cycles using an artificial graduated estrogen/progesterone replacement regimen; 3) Donor oocyte/recipient cycles using a graduated estrogen/progesterone replacement regimen.
Materials and Methods

Endometrial echo pattern was evaluated transvaginally (TV) on the day of human chorionic gonadotropin (hCG) injection in women who had IVF-ET and on the day prior to starting progesterone, in the women who had frozen ETs with their own oocytes, and also oocyte recipients who had fresh ETs. Endometrial echo patterns were evaluated by grading the pattern, as previously described, by visualizing the endometrium perpendicular to the sound beam in the longitudinal axis. A woman whose endometrial axis was not perpendicular to the sound beam and could not be accurately optimized and graded TV was scanned transabdominally (TA) to assure accurate grading [6].

IVF-ET cycles, frozen ET and donor oocyte cycles were included from 1/1/1997 to 7/31/09. For IVF-ET cycles all types of controlled ovarian hyperstimulation regimens were included, e.g., luteal phase leuprolide acetate, or gonadotropin releasing hormone antagonist (GnRH-ant) protocols with either cetrotelix or ganirelix. Women with diminished oocyte reserve were treated with mild ovarian stimulation protocols as previously described [7]. The minority of frozen ET cycles that did not use a graduated oral/vaginal estradiol replacement protocol but instead were transferred in a natural cycle were excluded.

To determine if women with diminished oocytes may have the need for a more “perfect” endometrium to achieve better pregnancy rates, women who had controlled ovarian hyperstimulation (COH), oocyte retrieval and ET were evaluated according to whether they had normal oocyte reserve (day 3 serum FSH ≤ 11 mIU/ml) vs diminished oocyte reserve (with serum FSH ≥ 12 mIU/ml). For this study, in contrast to the earlier ones, there was no requirement to attain a 10 mm endometrial thickness. Only the patient’s first cycle in each of the treatment categories was used in the analysis.

Analysis of variance (ANOVA) was used to evaluate mean values of potential variables, e.g., age, baseline serum FSH, estradiol (E2), the day of oocyte retrieval, number of oocytes retrieved, and number of embryos transferred. Chi-square analysis was used to compare pregnancy and implantation rates.

Results

The viable (live fetus at 12 weeks) and live delivered pregnancy rates in COH IVF-ET cycles in women with normal oocyte reserve and the live delivered implantation rate per embryo are shown in Table 1. Comparison of mean values was performed by analysis of variance. There were no significant differences or clinically important trends noted when comparing TL vs IE (chi-square analysis) echo patterns in women aged ≤ 40 with normal oocyte reserve undergoing IVF-ET.

The pregnancy and implantation rates in women with diminished oocyte reserve following IVF-ET are shown in Table 2. Although there was a significant difference in the endometrial thickness between the two groups, this did not appear to effect outcome since there was no significant difference in pregnancy or implantation rates according to pattern.

The pregnancy and implantation rates for frozen ET are shown in Table 3. Again there was a significant difference in endometrial thickness (but opposite of finding in IVF-ET with diminished oocyte reserve) but no significant differences in pregnancy rates or live delivered pregnancy rates according to TL or IE pattern.

The pregnancy rates and live delivered implantation rates for recipient cycles (fresh transfers only) are shown in Table 4. As seen with COH, IVF-ET cycles in women with normal and independently in women with diminished oocyte reserve, and in frozen ET (Tables 1-3), no differences in pregnancy or implantation rates were found comparing TL vs IE patterns in the late proliferative phase (chi-square analysis) for donor oocyte recipients.

### Table 1. — Comparison of potential confounding factors and pregnancy rates by endometrial echo pattern in women aged ≤ 40 with normal oocyte reserve following IVF-ET.

<table>
<thead>
<tr>
<th></th>
<th>TL</th>
<th>IE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33.8 ± 4</td>
<td>34.1 ± 3.6</td>
<td>0.28</td>
</tr>
<tr>
<td>Mean baseline FSH</td>
<td>6.0 ± 2.5</td>
<td>6.4 ± 2.2</td>
<td>0.21</td>
</tr>
<tr>
<td>Mean serum E2 day of retrieval</td>
<td>1970.2 ± 1082.4</td>
<td>2058 ± 1032.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Endo thickness day of hCG (mm)</td>
<td>10.9 ± 2.4</td>
<td>10.7 ± 2.9</td>
<td>0.25</td>
</tr>
<tr>
<td># oocytes retrieved</td>
<td>11.7 ± 7.2</td>
<td>12.1 ± 7.3</td>
<td>0.43</td>
</tr>
<tr>
<td># embryos transferred</td>
<td>2.6 ± .8</td>
<td>2.7 ± .9</td>
<td>0.08</td>
</tr>
<tr>
<td>Viable pregnancy rate</td>
<td>577/1456</td>
<td>82/232</td>
<td>0.24</td>
</tr>
<tr>
<td>Delivered pregnancy rate</td>
<td>539/1456</td>
<td>77/232</td>
<td>0.29</td>
</tr>
<tr>
<td>Delivered implantation rate</td>
<td>22.1%</td>
<td>18.9%</td>
<td>0.08</td>
</tr>
</tbody>
</table>

### Table 2. — Comparison of potential confounding factors and pregnancy rates by endometrial echo pattern in women aged ≤ 40 with diminished oocyte reserve.

<table>
<thead>
<tr>
<th></th>
<th>TL</th>
<th>IE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>36.0 ± 3</td>
<td>36.3 ± 3.2</td>
<td>0.55</td>
</tr>
<tr>
<td>Mean baseline FSH</td>
<td>20.2 ± 11.7</td>
<td>18.2 ± 8.9</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean serum E2 day of retrieval</td>
<td>977.3 ± 834.5</td>
<td>964.6 ± 877.6</td>
<td>0.93</td>
</tr>
<tr>
<td>Endo thickness day of hCG (mm)</td>
<td>9.9 ± 2.2</td>
<td>9.1 ± 2.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean # oocytes retrieved</td>
<td>4.6 ± 4.4</td>
<td>5.6 ± 4.9</td>
<td>0.18</td>
</tr>
<tr>
<td># embryos transferred</td>
<td>2.1 ± 1</td>
<td>2.1 ± 1</td>
<td>1.0</td>
</tr>
<tr>
<td>Viable pregnancy rate</td>
<td>109/361</td>
<td>10/40</td>
<td>0.62</td>
</tr>
<tr>
<td>Delivered pregnancy rate</td>
<td>104/361</td>
<td>10/40</td>
<td>0.75</td>
</tr>
<tr>
<td>Delivered implantation rate</td>
<td>17.5%</td>
<td>14.6%</td>
<td>0.62</td>
</tr>
</tbody>
</table>

### Table 3. — Comparison of potential confounding factors and pregnancy rates by endometrial echo pattern in frozen ET cycles.

<table>
<thead>
<tr>
<th></th>
<th>TL</th>
<th>IE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34.7 ± 5.8</td>
<td>36.0 ± 6.4</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean Endo thickness (mm)</td>
<td>9.9 ± 2.2</td>
<td>10.4 ± 2.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td># embryos transferred</td>
<td>2.8 ± .8</td>
<td>2.8 ± .8</td>
<td>NS</td>
</tr>
<tr>
<td>Viable pregnancy rate</td>
<td>676/1854</td>
<td>131/390</td>
<td>0.30</td>
</tr>
<tr>
<td>Delivered pregnancy rate</td>
<td>628/1854</td>
<td>121/390</td>
<td>0.31</td>
</tr>
<tr>
<td>Delivered implantation rate</td>
<td>16.7%</td>
<td>16.8%</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Effect of triple line vs isoechogenic endometrial texture on pregnancy outcome following embryo transfer according to use of et al.

Discussion

The starting date for this retrospective study was chosen because this was the initiation of improved pregnancy rates for our IVF center. Also there was clearly no overlap in patients with the previous studies.

This study is by far the largest one of its kind. It clearly shows that the presence of an isoechogenic pattern on the day of hCG injection should not influence the decision as to whether to transfer the embryos fresh or cryopreserve them. This applies even to women with diminished oocyte reserve.

Though the aforementioned study by Noyes et al. also found no live pregnancies with an HH pattern in women exposed to DES in utero, they failed to find any adverse effect of the HH pattern in those women not exposed to DES in utero. Since our policy is to freeze all embryos and defer fresh transfer if an HH pattern is present in the late proliferative phase, we unfortunately could not answer the question as to whether this pattern is still a poor prognostic factor for pregnancy in the modern IVF era.

Hopefully this study might influence another IVF center whose policy is not to cryopreserve all embryos with an HH pattern to perform a similar retrospective comparison to see if in the modern IVF era the HH pattern is still associated with a poor prognosis.

References


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Frequency of endometriosis and adenomyosis in patients with leiomyomas, gynecologic premalignant, and malignant neoplasias

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Summary

Objective: This study investigated the association between gynecological neoplasms, endometriosis, and adenomyosis in women who underwent surgical treatment for gynecological cancer and uterine leiomyoma during reproductive years or after menopause.

Materials and Methods: Information was collected from patient records from the Hospital’s database from 1985 to 2007. The study included 502 women, of which 375 were premenopausal and 132 were postmenopausal. Results: A significant association was observed between the occurrence of adenomyosis in cancer in women with four or more pregnancies, and in women aged over 40 years ($p < 0.0001$). The frequency of adenomyosis was significantly higher than the frequency of endometriosis for cancer in two sites ($p = 0.0419$) or for leiomyomas ($p < 0.0001$). Conclusion: Therefore adenomyosis is more frequently found than endometriosis in women with leiomyomas or cancer in two sites in premenopausal women, and clinicians need to be aware of patients with adenomyosis and the risk of cancer.

Key words: Adenomyosis; Endometriosis; Cancer association; Leiomyoma.

Introduction

Malignant transformation of endometriosis was first described in 1925 by Sampson and supported by additional evidence in further studies [1-10]. This transformation is described in various organs but occurs mainly in the ovaries [11]. Women with endometriosis have an increased risk for some types of malignancies and up to one percent develop cancer associated with these lesions [5]. Those who are diagnosed early in life and whose affected site is the ovary, have the highest risk for cancer in that organ [4].

Ovarian neoplasms, mainly endometrioid adenocarcinomas and clear cell carcinomas, are associated with the presence of ectopic endometrial tissue [1, 2]. The association between endometriosis and ovarian adenocarcinomas was supported by a study using a combination of clinical, pathological, and molecular data [3]. Data from molecular genetics and genetic aberrations, such as mutation type or loss of heterozygosity mutation, also provide evidence that endometriosis is a precursor of ovarian cancer [6, 8-10].

Ectopic endometrium undergoes malignant transformation, but how often this transformation occurs remains unknown [7]. Nonetheless, the causal relationship between endometriosis and specific types of ovarian cancer should be recognized [12]. Women with cancers associated with endometriosis probably represent a different group of patients than those who have traditional ovarian cancer and may require different therapeutic treatments [5, 13]. The elucidation of the true relationship between endometriosis and the development of malignancies is likely to have an impact on treatment options and follow-up of these patients [4].

The relationship between endometriosis and cancer is well-described in literature. The associative frequency of these conditions is an important issue requiring further information regarding this association and its impact in addressing cases with a known association. Therefore, the objective of this study was to verify the presence of endometriosis and adenomyosis in patients undergoing surgery for gynecological cancer and uterine leiomyoma.

Materials and Methods

Patient selection

A retrospective study from 1985 to 2007 was carried out in the Oncological Research Institute (IPON), Discipline of Gynecology and Obstetrics of the University of Triângulo Mineiro. Data were collected from medical records of patients who underwent surgical treatment for gynecological cancer or uterine leiomyoma in the University Hospital of the Federal University of Triângulo Mineiro.

The study included 502 women (375 premenopausal and 132 postmenopausal). Surgical procedures were performed and the presence or absence of adenomyosis and/or endometriosis-associated malignancy was evaluated.

Diagnosis was leiomyoma and gynecologic cancer for 37 and 85 surgeries, respectively. Wertheim-Meigs surgery was used for cervical cancer. Total hysterectomy, bilateral salpingooophorectomy, omentectomy, multiple biopsies, and pelvic lymphadenectomy were performed for endometrial or ovarian cancer. Patients with cervical intraepithelial neoplasia (CIN) 3 were submitted to a hysterectomy when there was no technical condition for conization. All surgeries were performed by a gynecologist or gynecologic oncologist with experience.
including one or two residents in gynecology at the Discipline of Gynecology and Obstetrics. Age, parity, menarche, and menopausal age, use of oral contraceptives at diagnosis, family history of cancer, surgical procedure, staging of endometriosis, cancer staging, and main symptoms were recorded.

Statistical analysis and ethical approval
Data were analyzed using GraphPad Instat software Chi-square ($\chi^2$) and Fisher tests were used for statistical analysis with the significance level set at $p < 0.05$.

The Research Ethics Committee of Federal University of Triângulo Mineiro approved this research.

Results
The mean age of premenopausal patients was 39.06 years (range 13 to 55). The mean age of menopausal women was 58.67 years (range 42 to 79). The main symptoms presented by premenopausal patients were: increased abdominal size (9.33%), abdominal pain (30.4%), metrorrhagia (37.07%), menstrual irregularity (7.2%), no symptoms (51%), and other symptoms (2.4%).

The main symptoms presented by postmenopausal patients were: increased abdominal size (18.18%), abdominal pain (28.79%), postmenopausal bleeding (29.55%), and no symptoms (23.48%).

Data analysis: premenopausal patients
Adenomyosis was found in 79 (21.07%) of 375 patients. The age with the highest association of cancer with adenomyosis was 41 - 50 years with 79.75% of cases (63 / 79). Patients under 30 years of age showed no association. A significant association was observed between the occurrence of adenomyosis in cancer in women with four or more pregnancies, and in women aged over 40 years ($p < 0.0001$). The association of adenomyosis and cancer was more frequent among women not using oral contraceptives (86.08% of cases; 68 / 79) and among those with a negative family history (83.54% of cases; 66 / 79 (Table 1).

Endometriosis was seen in 26 (6.93%) of 375 women, and in 12 / 26 (46.15%) of women aged 41-50 years. The association in nulliparous women was 30.77% (8/26). Patients with one to three pregnancies were 50% of cases (13 / 26). Endometriosis associated with cancer predominated in patients with no family history of cancer (65.38% of cases; 17 / 26) and who were not using oral contraceptives at the time of surgery (80.77% of cases; 21 / 26) (Table 2). Endometriosis stagings (American Society for Reproductive Medicine, 1996) were Stage I: 42.31%; Stage II: 26.92%; Stage III: 11.54%; Stage IV: 3.85%; other sites: 15.38%.

Adenomyosis was associated with 17.14% of uterine cervical cancers and 2.5% of ovarian cancers. No endometrial cancers were associated with adenomyosis and endometriosis. Endometriosis was associated with 4.17% of ovarian cancer cases, and 5.71% of all cervical cancer cases. In patients with leiomyoma, adenomyosis was present in 32.62%, and endometriosis in 9.09%. For cancer at two sites, adenomyosis was found in 30% of adenomyosis cases and 6.67% of endometriosis cases.
The histological subtypes and staging found in menacme were: 28.58% CIN 3 (Stage 0); 35.72% uterine cervical squamous cell carcinoma (Stages IA2, IB1, and IB2); 7.14% uterine cervical villoglandular adenocarcinoma (Stage IB1); 7.14% uterine cervical endometrioid adenocarcinoma (Stage IB2); 7.14% borderline ovarian serous cystadenoma (Stage IA); 7.14% borderline ovarian Brenner tumor (Stage IC); and 7.14% ovarian granulosa cell tumor (Stage IA).

Data analysis of menopausal women

Adenomyosis was present in 15 of 132 (11.36%) of postmenopausal women. An association between adenomyosis and cancer was found in 66.66% in the age group 41 - 60 years (10/15). Women with four or more pregnancies were 9 of 15 cases (60%), while only one was nulliparous (6.67%). No patients had used oral contraceptives and 86.67% (13 / 15) had no family history of cancer (Table 4). Only two cases of endometriosis were seen among 132 patients (1.52%) (Table 5). The stagings of endometriosis (American Society for Reproductive Medicine, 1996) in postmenopausal women were Stage II: 50%; other sites: 50%.

Uterine cervical cancer and ovarian cancers were associated with adenomyosis in 22.22% and 5% of cases, and with endometriosis in 18% and 60% cases, respectively. No endometrial cancer was associated with adenomyosis and endometriosis. Endometriosis was associated with only one (20%) case of leiomyoma and one (21%) case of cancer in two sites (Table 6). Leiomyomas and cancer in two sites were associated with adenomyosis in 30% and 9.52% cases, respectively, and associated with endometriosis in 5% and 4.76% cases, respectively.

Histological subtypes and staging found in menopausal women were: 54.55% CIN 3 (Stage 0); 36.36% uterine cervical adenocarcinoma (Stages IB2 and IIA); and 9.09% ovarian serous cystadenocarcinoma (Stage IIC).

Discussion

Adenomyosis is a common benign disease of the uterus that is seen in 15% - 30% of histopathological evaluations of hysterectomy [14]. In this study, the frequency of adenomyosis in the patient group was 21.07% for patients of reproductive age and 11.36% for menopausal patients. The exact incidence of endometriosis was unknown, because diagnosis depended on surgical procedure. Approximately 3% to 10% of women of reproductive age and 2% to 5% of postmenopausal women have endometriosis [15, 16]. In this present study, the frequency of endometriosis in patients with gynecological cancer was 6.93% for patients of reproductive age and 1.52% of menopausal patients.

Age, multiparity, high levels of follicle-stimulating hormone (FSH), prolactin, and smoking are risk factors for adenomyosis [17]. This study demonstrated a significant association between the occurrence of adenomyosis in cancer in women with one to three pregnancies, and in women over 40 years of age (p < 0.0001) in the premenopausal group. Endometriotic lesions can predispose to clear cell and endometrioid ovarian cancers; advancing age and the size of endometriomas were independent pre-

| Table 4. — Frequency of adenomyosis related to age, parity, oral contraceptives use, cancer family history, and menarche age in menopausal patients.
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| Table 5. — Frequency of endometriosis related to age, parity, oral contraceptives, cancer family history, and menarche age in menopause patients.
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<td>49</td>
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| Table 6. — Adenomyosis and endometriosis by site of gynecologic premalignant and malignant neoplasia in menopausal women.
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<th>%</th>
<th>Endometriosis</th>
<th>Endometriosis</th>
<th>Total</th>
<th>%</th>
<th>p</th>
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<td></td>
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<td>5</td>
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<td>Total</td>
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<td>2</td>
<td>132</td>
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dictors of development of ovarian cancer among women with ovarian endometrioma [18].

Endometriosis is a common disease that affects quality of life and fertility, and its prevalence is increasing [19]. Although considered a benign disorder, it has some malignant characteristics in terms of progression such as invasion and metastasis, which often affect other organs. Immune response abnormalities and inflammation have been shown in women with endometriosis, predisposing them to cancer and infections [20]. Cancer may be promoted by growth factors, cytokines, and inflammatory mediators that reach the ovarian epithelium during retrograde menstruation. Endocrine-disrupting environmental toxins that modify the inflammatory process can be associated with endometriosis [21].

Endometriosis is a progressive disease that is often debilitating because it causes chronic pelvic pain and infertility [22, 23]. The frequency of endometriosis associated with a neoplasm was 6.93% in premenopausal infertility [22, 23]. The frequency of endometriosis in endometrioid and clear cell types has been demonstrated to be higher than in the general population [16, 24]. Genetic alterations in ovarian cancers and adjacent endometriosis, such as p53 and bcl gene mutations, show a possible malignant genetic transition. Endometriosis is associated with chronic inflammation and cytokines can induce or repress their own synthesis and cause unregulated mitotic division, growth, differentiation, migration, or apoptosis similar to malignant mechanisms [16]. Gemmil et al. demonstrated a higher prevalence of recurrent upper respiratory or vaginal infections, melanoma, and ovarian cancer in patients with endometriosis than in the general population [25].

Adenomyosis is characterized by the presence of an ectopic endometrium with or without hyperplasia of the surrounding myometrium. Adenomyosis and leiomyomas can usually coexist [17, 26] and patients with these conditions may have more chronic pelvic pain [27]. The relationship between endometriosis and cancer is shown in several studies, but only two case studies have reported adenocarcinomas developing within adenomyosis [28, 29].

Adenomyosis associated with neoplasia was significantly higher than endometriosis in premenopausal patients with two-site tumors (p = 0.0419) and leiomyomas (p < 0.0001). Ovarian cancers were more frequently associated with endometriosis than with adenomyosis, but this was not significant. This fact could be explained by the small number of cases in this study or because they included benign neoplasias.

The role of genetic alterations has been discussed in relation to putative oncogenes and tumor suppressor genes that may be involved in endometriosis [30]. Mucin 1 (MUC1) glycoprotein is present in eutopic human endometrial glands, and in ectopic lesions of ovarian endometriosis, and is overexpressed in endometrioid and clear cell ovarian tumors. Changes in MUC1 expression in endometriosis could promote adaptive anti-MUC1 immunity that might play a role in malignant progression [31]. Pathological changes can reflect genetic alterations; in clear cell carcinoma, K-ras mutations were associated with malignant transformation of clear cell carcinoma [32]. Bischoff et al. demonstrated that perturbations of chromosome 17 in general and the p53 locus in particular, occur frequently in severe/late stage endometriosis [33]. Some genetic alterations that induce p53 mutations in endometriosis can affect malignant transformation of endometriosis in ovarian clear cell carcinoma [34]. Amemiya et al. demonstrated that K-ras mutations and microsatellite instability are associated with malignant transformation from endometriosis to ovarian endometrioid carcinoma [35]. Adenomyosis might be considered a special form of endometriosis. However, uterine adenomyosis rarely responds to hormonal therapy and its cure usually requires hysterectomy. Oehler et al. suggested that mutation-related silencing of estrogen responsiveness might render endometriotic cells resistant to hypoestrogenic conditions, accounting for failure of estrogen-ablative therapy in adenomyosis [36]. In contrast, Wang et al. found no positive recurrent gene copy number alterations in 25 cases of pathologically-proven adenomyosis utilizing comparative genomic hybridization; they concluded that genetic changes might be extremely rare in adenomyosis, or comparative genomic hybridization was not sensitive enough to detect candidate genes [37].

The present study detected a significantly higher frequency of adenomyosis, compared to endometriosis in cancer in two sites, which has not yet been reported by other studies. Further research is needed to better understand the relationship of genetic alterations in adenomyosis predisposing to cancer, which is already well-established in cases of endometriosis.

Conclusion

The authors demonstrated that adenomyosis is more frequently found than endometriosis in women with leiomyomas or cancer in two sites in premenopausal women. This emphasizes the need for clinicians to be aware of patients with adenomyosis and the risk of developing cancer, and new studies on neoplasms and adenomyosis are needed to elucidate this relationship.

Acknowledgments

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References


Placental apoptosis in preeclampsia, intrauterine growth retardation, and HELLP syndrome: An immunohistochemical study with caspase-3 and bcl-2

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Introduction

Hypertensive pregnancy disorders like preeclampsia, hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome, as well as intrauterine growth retardation (IUGR) are still some of the most common causes of maternal-fetal morbidity and mortality [1]. Although the etiology of these pregnancy disorders remains unclear, it is generally believed that impairment of trophoblastic invasion plays a key role in the etiology. Impaired/shallow interstitial trophoblastic invasion leads to maladaptation of uteroplacental arteries and then a placental factor is released into maternal circulation causing maternal endothelium damage [2, 3].

Apoptosis, a form of programmed cell death, of trophoblasts has been detected in normal human placentas [4, 5] and in placentas of preeclampsia, IUGR, and HELLP syndrome [6-8].

In preeclampsia or IUGR, changes in apoptosis regulation of villous and extravillous trophoblasts results in altered trophoblastic invasion and then shedding into maternal circulation causing clinical symptoms [3]. However, in the literature the assessment of placental apoptosis differs due to different techniques and methods of quantification [9, 10].

Molecular mechanisms leading to apoptosis are complex and include signal transduction pathways that trigger or inhibit apoptosis [11]. The trigger of apoptosis depends on the balance between pro- and anti-apoptotic proteins.

Caspase 3 is a member of the cysteine-aspartic acid protease (caspase) family and exists as a zymogen (pro-caspase) in almost all cells and is involved in the development of apoptotic cell death [11].

Bcl-2 is an anti-apoptotic protein expressed in the trophoblast layer of placental villi and high expression of bcl-2 in syncytiotrophoblasts would protect this key layer of placental villi from apoptosis [12].

As a result there is a controversy about the role of placental apoptosis in hypertensive pregnancy disorders and IUGR. Increased placental apoptosis [4, 13,14] and reduced placental apoptosis [6, 15] in hypertensive pregnancy disorders and IUGR have been reported in the literature.

In this study, the authors have investigated the expression of caspase-3 and bcl-2 in the placental tissue samples of pregnancies complicated with preeclampsia, HELLP syndrome, and IUGR indicating increased placental apoptosis.

Materials and Methods

This was a prospective case-control study which was conducted on 50 pregnant women between December 2006 and August 2007 at Dr. Zekai Tahir Burak Women Health Research and Education Hospital, Ankara, Turkey. Placental tissue samples were obtained from 15 pregnancies complicated by preeclampsia, 15 pregnancies with normotensive IUGR, five pregnancies with HELLP syndrome, and 15 gestational age-matched normotensive pregnancies without intrauterine infection as a control group. The placental expression of caspase-3 and bcl-2 has been investigated by immunohistochemical staining.

Results:

Caspase-3 immunostaining score was significantly higher in each group when compared with the control group (p = 0.002). However there was no statistically significant difference with bcl-2 immunostaining in each group when compared with the control group.

Conclusions: Apoptotic marker caspase-3 is significantly increased in the villous trophoblasts of patients with preeclampsia, HELLP syndrome, and IUGR indicating increased placental apoptosis.

Key words: Caspase-3; Bcl-2; Apoptosis; Preeclampsia; IUGR; HELLP syndrome.
intrauterine infection as a control group. Written informed consent was obtained from all subjects and the study protocol was approved by the Institutional Ethics Committee.

Preeclampsia and HELLP syndrome were defined according to the National High Blood Pressure Education Program [16]. IUGR was defined as either an ultrasound (US) estimate of fetal weight or an US measurement of the fetal abdomen < 5th percentile for gestational age, confirmed at delivery (birthweight < 5th percentile for age and gender) and not associated with aneuploidy, structural anomalies, or congenital infection.

Placental samples were taken from both vaginal deliveries and cesarean sections. Analysis was performed in two placental samples from the central part of each placenta per patient. The samples were cut into small pieces (2 x 2 x 2 cm) and rapidly fixed in 10% formalin for 24 hours at room temperature. After fixation, samples were embedded in paraffin wax. From these blocks, three 5 μm sections were cut for each placental sample and mounted on slides. These slides were stained with hematoxylin and eosin and at least 20 fields were examined per slide. Different cell types in the placenta were identified under light microscope and selected for immunohistochemistry.

Immunohistochemistry for caspase-3 and bcl-2 was performed using a combination of the streptavidin-biotin-peroxidase method and microwave antigen retrieval on formalin-fixed paraffin embedded tissues. After deparaffinization, samples were treated with 10% hydrogen peroxidase in filtered water to block endogenous peroxidase activity. To attain antigen, slides were treated with 10 mmol/l citrate buffer (pH: 6.8) for 10 min. After preincubation with Ultra V block (Lab Vision) for 20 min, samples were incubated with primary antibody for an hour at room temperature for caspase-3 (rabbit polyclonal antibody, CPP32.7 ml, Neomarkers) and bcl-2 Ab-1 (mouse monoclonal antibody, Clone 100/D5.7 ml, Neomarkers). The positive controls were lymphoid tissue for caspase-3 and lymphoma for bcl 2α. Negative control was achieved by the same way without application of the primary antibody.

The sections were examined by the pathologist at high power (x200) in a blinded design. Immunohistochemical evaluation was carried out in the epithelium of the trophoblastic cells. The percentage of positively stained area to the total area of villous trophoblasts in each section was calculated [17]. The staining score for caspase-3 and bcl-2 was labeled as ‘0’ for no immunostaining, ‘1+’ for weak and focal immunostaining, ‘2+’ for weak and diffuse immunostaining, ‘3+’ for strong and diffuse immunostaining. The results of all four scores from each case were summarized to achieve the final immunostaining result.

Statistical analysis of the data was carried out by using the SPSS 13. To analyze the correlation between the scores from each group, the Kruskal-Wallis test was used. Differences were accepted as significant for $p < 0.05$.

Results

Clinical characteristics of patients are summarized in Table 1. There were no significant differences in maternal age and parity between the study groups. However gestational age and fetal weight were significantly lower in the HELLP group than the control group ($p < 0.05$).

Immunohistochemical analysis of caspase-3 and bcl-2 in the placental tissue samples of patients are shown in Table 2. Examples of placental staining with caspase-3 and bcl-2 are shown in Figures 1 and 2, respectively.
Expression scores of caspase-3 immunostaining were 1.0 ± 0.4 in the preeclamptic group, 1.2 ± 0.4 in the IUGR group, 1.2 ± 0.5 in the HELLP group, and 0.5 ± 0.5 in the control group.

As a result, caspase-3 immunostaining score was significantly higher in each group when compared with the control group (p = 0.002). When the authors compared each group with the control group separately, each group stained more strongly with caspase-3 than the control group (Table 2).

Bcl-2 immunostaining scores were 1.9 ± 0.8 in the preeclamptic group, 2.1 ± 1.0 in the IUGR group, 2.2 ± 0.8 in the HELLP group, and 2.3 ± 0.5 in the control group. There was no statistically significant difference with bcl-2 immunostaining in each group when compared with the control group (p = 0.518) (Table 2).

**Discussion**

Altered uteroplacental blood flow is the key event of abnormal pregnancy in either preeclampsia or IUGR [2]. A recent model for the pathogenesis of preeclampsia describes a process by which a placental factor is released into the maternal circulation causing damage to maternal endothelium with systemic inflammatory response [18].

Maternal systemic inflammatory response also occurs in normal pregnancy but is more severe in preeclampsia [19]. Placental factor seems to be the syncytiotrophoblastic debris released into maternal circulation as a result of syncytiotrophoblastic apoptosis, which is a part of normal cell turnover and repair [4, 10].

Apoptosis is a descriptive term for the unique morphology of cell suicide and may be a part of normal physiology or secondary to pathological conditions [20]. Apoptosis of trophoblasts has been detected in normal human placentas [4, 5] and in placentas of preeclampsia, IUGR, and HELLP syndrome [6-8].

In the literature the assessment of placental apoptosis differs due to different techniques and methods of quantification [9, 10] and as a result there is a controversy about the role of placental apoptosis in hypertensive pregnancy disorders and IUGR.

Although increased placental apoptosis in abnormal pregnancies is generally accepted [4, 5, 8, 13, 14], there are also some studies indicating reduced placental apoptosis [6, 15] in hypertensive pregnancy disorders and IUGR.

In the present study, apoptotic marker caspase-3 is significantly increased in the villous trophoblasts of patients with preeclampsia, HELLP syndrome, and IUGR compared to a control group. However placental staining of anti-apoptotic marker bcl-2 showed no statistically significant difference among groups.

In the literature, there are similar results indicating increased placental apoptosis in pregnancies complicated with IUGR and preeclampsia as in this study. Aban et al. demonstrated increased placental apoptosis shown by M30 and caspase-3 staining accompanied by increased NF-κB and decreased bcl-2 expression in pregnancies complicated with IUGR and preeclampsia [13]. Ishihara et al. have shown increased apoptosis throughout fas antigen and bcl-2 in human term placenta complicated by either preeclampsia or IUGR [14]. Another report studying the placental bed of pregnancies complicated by preeclampsia demonstrated widespread apoptosis with a decrease in bcl-2 expression [8].

However there have also been some studies reporting decreased placental apoptosis in pregnancies complicated with IUGR and preeclampsia. Kadyrov et al. demonstrated decreased apoptosis in the extravillous trophoblasts in preeclamptic placentas using a M30 antibody [15] and another study by Stepan et al. showed that apoptotic mediators BNip3 and Nix are decreased in the villous trophoblast cells of patients with preeclampsia, HELLP syndrome and IUGR [6]. They explained the decreased apoptosis as a tolerance to chronic hypoxia in the placenta that could cause less trophoblast apoptosis as expected [6].

These apoptosis features of placenta are controversial because of methodological restrictions associated with limitations of human tissue investigations and animal studies. Basal plates of delivered placentas or curettage specimens have been used in most studies, but pathogenic events occur in the placental bed [2]. Placental bed biopsy
specimens are available only in a few groups obtained usually at the time of cesarean section [21, 22] or from hysterectomy uteri [15, 23]. Also pathogenesis of IUGR and preeclampsia occur in early stages of human pregnancy, so it is impossible to get placental bed biopsies at that time.

In case of animal experiments, it is also difficult to create a model similar to human pregnancy because of the variations in the trophoblastic invasion among experiment animals [24, 25]. Most of the placental-related studies have focused on nuclear changes that occur in the apoptotic process which are relatively subjective criteria. However apoptosis in human trophoblasts is a very complex process consisting of many signal transduction pathways, bcl-2 regulators, caspases, and substrates [20]. Also to decrease bias in the quantification of immunohistochemical staining in the placenta, more quantitative methods such as image analysis should be preferred.

As a result, a complex cascade of apoptosis in human placenta, difficulties in getting placental bed biopsies, small numbers of study materials, and variation of trophoblastic invasion in experimental animals comprise these controversies about apoptosis of human placenta in abnormal pregnancies. These methodological problems seem to be difficult to solve at present, however for future researches we should be aware of these limitations.

References


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Obstetric outcome in adolescence: a single centre experience over seven years

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Summary

Purpose of investigation: The aim of this study was to compare the obstetric outcome of adolescent pregnant women (aged 18) with the outcome of adult pregnant women who delivered in a tertiary university hospital. Materials and Methods: Delivery files from 2004 to 2011 were reviewed concerning age of the pregnant women, parity, gestational age, mode of delivery and birth weight of the neonates. Results: During the study period 119 (0.94%) out of 10,483 deliveries were performed in adolescent women. Caesarean section was the mode of delivery in 41 adolescent patients (34.45%), while the corresponding rate was 53.6% (5,556 cases) in adult pregnant women. The preterm labour rate in the adolescent group was 13.44% (16 cases) while in the adult group it was 21% (2,201 cases). The most frequent indication of caesarean section in the adults was previous caesarean section (21%). Discussion: In adolescent pregnancies the caesarean section rate was lower than in adult pregnancies. As far as the prevalent cause of caesarean section is concerned, it was repeat caesarean section for adults while in adolescents it was failure of labour to progress.

Key words: Adolescent; Obstetric outcome; Cesarean section; Vaginal delivery; Parity; Birth weight.

Introduction

It is considered that adolescent mothers are at increased risk of poor obstetric outcome due to the immaturity of their genital tract [1]. It is supported that adolescence may be associated with increased incidence of pregnancy complications such as preterm labour, intrapartum growth retardation and low birth weight resulting in increased caesarean section rates [2, 3]. Global differences in prenatal care programmes for teenage mothers and access to public or private hospitals with varying standards of obstetric management exist in developed and developing countries alike. Thus, conflicting data are reported in the literature regarding the obstetric outcome in adolescent mothers [4, 5]. The objective of this retrospective study was to present the obstetric outcome of pregnant women equal or younger than 18 years of age and compare it with an adult pregnant population (aged ≥ 19) who delivered in our tertiary department over a seven-year period. We also report the rate of preterm labour, the incidence of caesarean section and the most common indication for performing caesarean section in the two study groups.

Material and Methods

Medical records of 10,483 sequentially pregnant women who delivered in our single tertiary department from April 2004 to April 2011 were retrospectively reviewed. We classified them into two groups; the adolescent group was defined as teenage mothers ≤ 18 years of age and the adult group included mothers aged 19 years or older. Preterm delivery was defined as the birth of an infant before 37 weeks of gestation after premature rupture of membranes or preterm initiation of the active phase of labour with regular uterine contractions, at least three in 30 minutes, cervical effacement and dilatation.

Results

All births in the study were conducted by the same team of experienced midwives in the labour ward of our department with the advice and support of trainees and under the supervision of senior obstetricians. As a routine, continuous electronic foetal monitoring was provided for intrapartum foetal surveillance to detect foetal distress. Administration of oxytocin was used for labour induction or augmentation of dysfunctional labour according to the published obstetric protocols [6, 7]. The most common indications for caesarean delivery in our department included previous caesarean section, arrest of dilation or descent, non-reassuring foetal status, multiple gestations, malpresentations and several maternal-foetal conditions [8]. Caesarean section, in cases of labour arrest owing to cessation or inadequate uterine contractions for at least two hours without cervical change, was indicated only after failure of oxytocin augmentation [9].

In addition, diagnosis of cephalopelvic disproportion was attempted to be excluded in cases with a prolonged second stage of labour. Finally, the decision for caesarean section was usually made in consultation with the pregnant woman after a thorough discussion of the potential risks and benefits of the procedure.

From the total number of pregnant women (10,483), 119 pregnant adolescents were allocated to the first group (1.13%) and 10,364 pregnant adults (98.87%) to the second group during the study period. The mean age of teenage mothers was 15.93 ± 1.16 years old (range: 12-17) while in the adult group it was 29.90 ± 5.54 (range: 18-49).

In the former group, the vaginal delivery rate was 65.45% (78 adolescent mothers) while the corresponding rate in the latter group was 46.4% (4,808 adult mothers). Table 1 presents the indications for caesarean delivery in the teenage and adult groups.

The caesarean section rate among adolescent pregnant women was 34.45% (41 cases) while that for the adult pregnant women it was 53.6% (5556 cases). The major
indication for caesarean delivery was labour arrest or prolongation of second stage of labour in the adolescent pregnant group (26.82%), while repeat caesarean section was the most frequent (21%) indication for caesarean delivery in the adult pregnant group.

Caesarean section for preterm labour (< 37 weeks) occurred in 16 out of 119 (13.44%), in the adolescent group while in the adults it occurred in 2,201 out of 10,483 pregnancies (21%).

Furthermore, birth weights of the infants of these mothers did not differ significantly, and the mean birth weight in the adolescent mothers was 2,984 g (range: 1550-4560 g) while in the adult group it was 2,996.21 g (range: 440-4930 g) (Table 2).

Table 2. — Comparison of parity, gestational age, mode of delivery and birth weight between the adolescent and adult group.

<table>
<thead>
<tr>
<th>Maternal age (± SD) years</th>
<th>Adolescent group (n = 119)</th>
<th>Adult group (n = 10,364)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.93 ± 1.16</td>
<td>30.6 ± 0.68</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Primigravida</td>
<td>91.0% ± 40.0%</td>
<td>41.0% ± 50.0%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Gestational age</td>
<td>38.3 ± 2.16</td>
<td>37.8 ± 2.83</td>
<td>0.0286</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>78.55% ± 65.5%</td>
<td>48.03% ± 46.40%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>41 (34.45%)</td>
<td>55.6% (53.60%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Birth weight (± SD) g</td>
<td>2984.82 ± 508.38</td>
<td>2996.21 ± 690.51</td>
<td>0.8573</td>
</tr>
</tbody>
</table>

Indications for caesarean delivery was labour arrest or prolongation of second stage of labour, whereas the most common indication for cesarean section in adult mothers was history of previous caesarean section.

The classification of the indications of caesarean sections between labour arrest and prolongation of second stage of labour or cephalopelvic disproportion was a challenging task. The labour arrest for cesarean section was the second commonest indication in adolescent mothers while in adult mothers it was the fourth frequent cause.

The mean neonatal birthweight was 2,984 g in the teenagers while in the adults it was 2,996.21 g. In the relevant literature it has been suggested that the increased incidence of complications during labour in adolescent mothers younger than 18 years old is due to the immaturity of the female body and specifically of the bony pelvis [1]. However, such an observation is not confirmed by our findings. This could be attributed to the fact that the formation of the bony pelvis is completed as early as one year after menarche achieving 85% of its bone density [12, 13]. In that respect, it is unlikely that the pelvis of a teenage mother is not able to allow the vaginal delivery of a full term baby.

Furthermore, it has been observed in this study that the incidence of preterm labour in adolescent mothers is reduced compared to the incidence observed in adult mothers.

Since 1970 the fertility rate (number of livebirths per woman) has decreased throughout Europe and this is consistent with a reduction in the numbers of teenage pregnancies. The majority of the Western European countries have a low incidence of adolescent pregnancies which in Greece has been estimated to be around 10 per 1,000 births [14]. A possible explanation of this finding is the development and implementation of sexual awareness programmes and the widespread use of contraceptive measures.

Adolescent pregnancies constitute an important social problem and bear important consequences on the health of an adolescent mother and her newborn. Nevertheless, adequate perinatal care can significantly minimise the health risks of adolescent pregnancy and secure an uneventful antenatal course of pregnancy and delivery for the adolescent mother and her offspring.

**Discussion**

The incidence of caesarean section in adolescent mothers was lower compared to adults. This finding might be attributed to the conservative strategy that most obstetricians or physicians tend to adopt in this specific subgroup of pregnant women [10, 11]. This attitude is based on the notion that caesarean section at an early age might limit the number of future births [5]. The incidence of caesarean section in both groups was higher than that observed in the last decade. This is consistent with what has been reported in developed countries [12].

In our study, the most common indication for caesarean section in adolescent mothers was labour arrest and prolongation of second stage of labour, whereas the most common indication for cesarean section in adult mothers was history of previous caesarean section.

The classification of the indications of caesarean sections between labour arrest and prolongation of second stage of labour or cephalopelvic disproportion was a challenging task. The labour arrest for cesarean section was the second commonest indication in adolescent mothers while in adult mothers it was the fourth frequent cause.

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**Table 1. — Indications for caesarean section.**

<table>
<thead>
<tr>
<th>Indications</th>
<th>Adolescent group (n = 41)</th>
<th>Adult group (n = 5,556)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm labour</td>
<td>16 (39.0%)</td>
<td>2201 (39.6%)</td>
<td></td>
</tr>
<tr>
<td>Multiple gestation</td>
<td>511 (9.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labour arrest</td>
<td>11 (27.4%)</td>
<td>427 (7.6%)</td>
<td></td>
</tr>
<tr>
<td>Fetal distress</td>
<td>3 (7.2%)</td>
<td>275 (4.9%)</td>
<td></td>
</tr>
<tr>
<td>Repeat caesarean section</td>
<td>2 (4.8%)</td>
<td>1167 (21%)</td>
<td></td>
</tr>
<tr>
<td>IUGR</td>
<td>1 (2.4%)</td>
<td>210 (3.8%)</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>2 (4.8%)</td>
<td>126 (2.2%)</td>
<td></td>
</tr>
<tr>
<td>Antepartum hemorrhage</td>
<td>1 (2.4%)</td>
<td>105 (1.8%)</td>
<td></td>
</tr>
<tr>
<td>Congenital malformations</td>
<td>1 (2.4%)</td>
<td>35 (0.6%)</td>
<td></td>
</tr>
<tr>
<td>Malpresentations</td>
<td></td>
<td>35 (0.6%)</td>
<td></td>
</tr>
<tr>
<td>Breech presentations</td>
<td>3 (7.2%)</td>
<td>30 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>Fetal pathology</td>
<td>70 (1.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal pathology</td>
<td>1 (2.4%)</td>
<td>364 (6.5%)</td>
<td></td>
</tr>
</tbody>
</table>

**References**

Obstetric outcome in adolescence: a single centre experience over seven years


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The impact of socio-economic, lifestyle habits, and obesity in developing of pregnancy-induced hypertension in fast-growing country: global comparisons

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Summary

Objective: The aim of the study was to determine the prevalence and associated risk factors of pregnancy-induced hypertension (PIH) in the third trimester of Arab women and their neonatal outcome. Design: A prospective study. Setting: Women’s Hospital and Maternity Clinics. Subjects and Methods: The study was based on pregnant women in third trimester from the first week of January 2010 to April 2011. A total of 2,056 pregnant women, who had any kind of maternal complications, were approached and 1,608 women (78.2%) expressed their consent to participate in the study. A questionnaire covered variables related to socio-demographic factors, family history, medical history, maternal complications, and neonatal outcome. Multiple logistic regressions were used to describe the relationship between socio-demographic factors and PIH. Results: Pregnant women with Qatari nationality were 30% more likely to have PIH (Adj. OR 0.7; 95% CI 0.5-0.9, p = 0.03). Those living in villas were 50% more likely than those living in apartments (Adj. OR 0.5; 95% CI 0.3-0.9) and 40% more likely than those living in traditional houses (Adj. OR 0.6; 95% CI 0.4-0.8) to have PIH. The odds of PIH linearly increases with each decrease of 5,000 QAR in monthly income from > 20,000 to 10,000 (Adj. OR 1.2; 95% CI 0.7-2.1, Adj. OR 1.9; 95% CI 1.1-3.2, respectively) and then it starts decreasing from 10,000 to < 5,000 monthly income (Adj. OR 1.8;95% CI 1.1-3.1 and Adj. OR 1.3; 95% CI 0.7-2.7 respectively). The odds of PIH linearly increase with each five years increase in age among pregnant women from 30 to 45 years of age. A 10-fold increase in PIH odds was observed when body mass index (BMI) increased above 30 (obese) (Adj. OR 10.0; 95% CI 6.4-15.6). Pregnant women who had no history of previous abortion were 60% less likely than those who had positive history of previous abortion (Adj. OR 1.6; 95% CI 1.1-1.2; p = 0.007) to have PIH. The odds of PIH increases by 50% when women do not receive antenatal care (Adj. OR 1.5; 95% CI 1.1-2.1; p = 0.040). Conclusion: Qatar has a high prevalence of PIH compared to both regional and global rates. Maternal age > 30, increased BMI, previous abortion, lack of antenatal care, and physical activity were found to be significantly associated with increased risk of PIH in Arab women and could be potentially modifiable risk factors.

Key words: SES; Consanguinity; Life-style habits; Obesity; Gestational diabetes; Pregnancy-induced hypertension; Type of delivery.

Introduction

Pregnancy-induced hypertension (PIH) is one of the most common complications that occur during pregnancy and occurs in between 6-8% of pregnancies [1]. The two main conditions that comprise PIH are gestational hypertension and preeclampsia. Gestational hypertension is a more mild form of hypertension that does not result in many complications during pregnancy; nonetheless it has been found by a number of studies to predispose women to future chronic hypertension [2, 3]. Preeclampsia, on the other hand, is known to cause many detrimental complications to both the mother and the fetus, such as placental abruption, cerebrovascular accident, end-organ failure, disseminated intravascular coagulation [4] low birth weight [5], cesarean section deliveries [6], and even cases of neonatal and maternal death [7, 8].

A number of global studies have documented some of the common risk factors associated with PIH; these include nulliparity, maternal obesity, insulin resistance, multiple gestation, preexisting hypertension, and gestational diabetes mellitus (GDM) [1-3, 7-11]. While these studies have documented these general risk factors for PIH, to date, no study has been conducted in Qatar to determine the specific socio-demographic and biological risk factors associated with PIH.

It is particularly important to investigate the maternal factors associated with PIH in Qatar, as Qatar is currently undergoing rapid economic development. It now boasts one of the highest per capita incomes in the world. Such development has been accompanied by rapidly increasing rates of obesity [12]; type 2 diabetes mellitus, and the metabolic syndrome [13]. A number of studies conducted in Qatar have noted that women are particularly vulnerable and susceptible to developing each of these chronic illnesses [14]. This is an especially worrying trend for women of childbearing age, where there is a greater potential for poor obstetric and fetal outcomes.

Thus the aim of this study was to determine the prevalence and associated risk factors of pregnancy-induced hypertension in the third trimester of Arab women and their neonatal outcome.
Materials and Methods

This is a prospective hospital based study which was conducted among Arab pregnant women in the third trimester over a period from January 2010 to April 2011. The study was based on the logbook of the Women’s hospital which registers all pregnant women visiting antenatal clinics of the Women’s Hospital of the Hamad Medical Corporation. The research assistants screened the outpatient register of Women’s hospital during the study period and prepared a list of 2,056 Arab pregnant women above 28 weeks who came to the outpatient clinic with a complication in their pregnancy. A series of pregnant women with complications were taken consecutively from the register and included in the study sample. Only participants who agreed to participate were included in the study. A total of 2,056 pregnant women, who had any kind of maternal complications, were approached and 1,608 women (78.2%) expressed their consent to participate in the study; 448 women were excluded from the study due to incomplete questionnaires or did not want to respond to the questionnaire due to lack of time. Research Assistants screened medical files of the subjects for any queries about the pregnancy and neonatal complications.

In 2010, there were a total of 16,188 deliveries in the Women’s Hospital. Our study sample included 1,608 pregnant women which is 9.9% of the mothers who delivered. The study was approved by both the institutional review board (IRB) at Weill Cornell Medical College and Hamad Medical Corporation prior to commencing data collection. Each participant was provided with brief information about the study and was assured of strict confidentiality.

In the State of Qatar, cost-free health care is offered to all pregnant women in maternity clinics at the Primary Health Care (PHC) Center and Women’s Hospitals. Practically all pregnant women attend these clinics. During the study period, GDM screening in the PHCs and hospitals were based on assessment of risk factors, in accordance with national guidelines. Women were considered to be at risk if one or more of the following factors were present: age over 40 years, body mass index (BMI) of 25 kg/m² or greater, prior GDM, previous delivery of a macrosomic infant (birth weight > 4,500 g), glucosuria, and suspected fetal macrosomia in the current pregnancy. These women underwent diagnostic glucose tolerance testing, performed after an overnight fast, conducted by administering a 2-h, 75-g oral glucose tolerance test (OGTT). Diagnosis of GDM was set after one abnormal value in the OGTT.

Definitions. Age was considered as a continuous variable and dichotomized as under/over 40 years. Glucosuria during pregnancy was dichotomized as ever/never, and the number of abnormal values in the OGTT during pregnancy as one/several. Mean arterial pressure (MAP) after gestation week 36 was calculated using the formula MAP = diastolic blood pressure + (systolic blood pressure – diastolic blood pressure)/3.

Gestational hypertension was defined as systolic blood pressure exceeding 140 mm Hg or diastolic blood pressure exceeding 90 mm Hg, and preeclampsia was defined as proteinuria and blood pressure exceeding the aforementioned values after gestation week 20.

The primary outcomes were preeclampsia and gestational hypertension defined according to research criteria [1] using blood pressure (BP) recordings from prenatal visits, measurements during labor were not used to define pregnancy outcomes. Preeclampsia was defined as the new onset of hypertension (BP ≥ 140/90 mmHg) after 20 weeks of gestation in association with proteinuria, either ≥ 2 + by dipstick or ≥ 300 mg/24 h in the absence of urinary infection. Gestational hypertension was defined as the new onset of isolated hypertension that first appeared after 20 weeks of gestation [1]. Blood pressures were measured from subjects’ right arm using standard sphygmomanometers after they were seated at rest for 3-5 min. After selecting the proper cuff size on the basis of right midarm circumference, BP readings that coincided with the timing of the first (systolic) and fifth (diastolic) Korotkoff sounds were recorded. Hypertensive BP readings were repeated 5-10 min. later; if the subsequent readings were also elevated, they were recorded in the EMR.

A well-designed and pilot tested questionnaire was used to collect data. A face-to-face interview was conducted by qualified nurses using a validated self-administered questionnaire in the local language. The questionnaire covered socio-demographic characteristics of the pregnant women, family and medical history, type of maternal complication, and the pregnancy and neonatal outcome. A translated Arabic version of the questionnaire was revised by a bilingual consultant. The survey instrument was then tested on 100 randomly selected pregnant women from the list for the validity of the questionnaire. The investigators had made the necessary corrections and modifications after considering the minor differences and discrepancies that had been found during the pilot study.

Statistical analyses were performed using SPSS Version. 18.0 (SPSS Inc., Chicago, IL). Fisher’s exact test and chi-square analysis were performed to test for differences in the proportions of categorical variables between two or more groups. Student’s t-test (two-tailed) was used to determine the significance of difference between two continuous variables and confirmed by a non-parametric Mann-Whitney test. Multiple logistic regression analysis using the forward inclusion and backward elimination method was used to assess the relationship between dependent and independent variables and to adjust for potential confounders and orders the importance of risk factors (determinant) for the PIH. All multivariable analyses were adjusted for gestational age at the time of the first prenatal visit in order to account for variation in baseline BP and BMI that was associated with differences in the gestational age when they were measured. The level p < 0.05 was considered as the cut-off value for significance.

Results

Table 1 shows the prevalence and socio-demographic risk factors of PIH among pregnant women visiting the Women’s hospital.

Table 2 gives prevalence and biological risk factors of PIH among pregnant women visiting the Women’s hospital.

Table 3 shows multivariable analysis for predictors of PIH. Pregnant women with Qatari nationality were 30% more likely to have PIH (Adj. OR 0.7; 95% CI 0.5-0.9, p value 0.03). Those living in villas were 50% more likely than those living in apartments (Adj. OR 0.5; 95% CI 0.3-0.9) and 40% more likely than those living in traditional houses (Adj. OR 0.6; 95% CI 0.4-0.8) to have PIH. The risk of PIH linearly increases with each decrease of 5,000 QAR in monthly income from > 20,000 to 10-15,000 (Adj. OR 1.2; 95% CI 0.7-2.1, Adj. OR 1.9; 95% CI 1.1-3.2, respectively) and then it starts decreasing from 10,000 to < 5,000 monthly income (Adj. OR 1.8;95% CI 1.1-3.1, and Adj. OR 1.3; 95% CI 0.7-2.7, respectively).
Risk of PIH linearly increased with each five years increase in age among pregnant women from 30 to 45 years of age. The risk of PIH synergistically increases to 10-fold when BMI increases above 30 (obese) (Adj. OR 10.0; 95% CI 6.4-15.6). The risk of PIH among pregnant women who have no history of previous abortion is 60% less than those who have positive history of previous abortion (Adj. OR 1.6; 95% CI 1.1-1.2; p = 0.007). The risk of PIH increases by 50% when the women do not receive antenatal care (Adj. OR 1.5; 95% CI 1.1-2.1; p = 0.040).

Table 4 presents characteristics and comparison of differential risk of hypertensive disorders of pregnancy among Hispanic, Caucasian, and Qatari women. All variables showed statistically significant differences for the risk of PIH (p < 0.01), except for gestational weeks.

Discussion

Our study indicates a very high prevalence of PIH (17.4%) in comparison to other studies conducted in the region and globally (Table 5). Regional rates mentioned in published studies range between 2.32% in Iran [15] to 8.49% in Turkey [16]. Global rates on the other hand range from 7.5% in Brazil [17], from 6.3% to 10% in Canada [3], 13.9% in Northern Finland [18] and 3.6% in Singapore [19]. A possible explanation for the high rates found in our study could be attributed to the rising metabolic syndrome (MetS) epidemic [20] where T2DM and HPT rates are reaching alarming levels in the general population of Qatar [12-14]. Indeed a number of studies conducted in the USA [21, 22] have noted when comparing White and Black women, that Black women were more likely to have PIH and complications because Black women of reproductive age, are more likely to have a comorbidity or pre-existing medical condition, such as hypertension, diabetes, or obesity.
Table 3. — Multivariable analysis for predictors of pregnancy induced hypertension in Qatar (n = 1608).

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Adjusted OR (95% CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30 yrs (ref)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>1.1 (0.7-1.5)</td>
<td>0.025</td>
</tr>
<tr>
<td>35-39</td>
<td>1.6 (1.2-2.4)</td>
<td></td>
</tr>
<tr>
<td>40-45</td>
<td>1.5 (1.1-2.3)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25 (ref)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td>2.6 (1.6-4.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>10.0 (6.4-15.6)</td>
<td></td>
</tr>
<tr>
<td>Nationality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qatari (ref)</td>
<td>1</td>
<td>0.037</td>
</tr>
<tr>
<td>Non-Qatari</td>
<td>0.7 (0.5-0.9)</td>
<td></td>
</tr>
<tr>
<td>Housing condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villa (ref)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Traditional house</td>
<td>0.6 (0.4-0.8)</td>
<td>0.004</td>
</tr>
<tr>
<td>Apartment</td>
<td>0.5 (0.3-0.9)</td>
<td></td>
</tr>
<tr>
<td>Monthly income (QR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5,000</td>
<td>1.3 (0.7-2.7)</td>
<td></td>
</tr>
<tr>
<td>5,000-9,999</td>
<td>1.8 (1.1-3.1)</td>
<td></td>
</tr>
<tr>
<td>10,000-14,999</td>
<td>1.9 (1.1-3.2)</td>
<td>0.049</td>
</tr>
<tr>
<td>15,000-20,000</td>
<td>1.2 (0.7-2.1)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20,000 (ref)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Previous abortion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.6 (1.1-2.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>No (ref)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>APH§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.0 (1.3-3.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>No (ref)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ante partum care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (ref)</td>
<td>1</td>
<td>0.040</td>
</tr>
<tr>
<td>No</td>
<td>1.5 (1.1-2.1)</td>
<td></td>
</tr>
</tbody>
</table>

ref = reference category, § APH = anti partum hemorrhage, Adjusted OR (95% CI) = Adjusted odds ratios based (95% confidence interval), *Two sided p value based on z-2 log likelihood statistics. Model based on backward logistic regression. Model goodness of fit tested with Hosmer-Lemeshow goodness of fit test.

Table 4. — Characteristics and comparison of differential risk of hypertensive disorders of pregnancy among Hispanic, Caucasian, and Qatari women.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hispanic n = 863</th>
<th>Caucasian n = 381</th>
<th>Qatari n = 1,608</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>22.6 ± 5.2</td>
<td>30.0 ± 5.4</td>
<td>32.2 ± 6.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 5.4</td>
<td>24.5 ± 4.7</td>
<td>27.8 ± 5.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>107 ± 11</td>
<td>113 ± 11</td>
<td>125 ± 8.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65 ± 8</td>
<td>71 ± 8</td>
<td>77 ± 8.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>Yes</td>
<td>46</td>
<td>37</td>
<td>4.9</td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>39</td>
<td>95.1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Gestational diabetes (%)</td>
<td>2.6</td>
<td>2.5</td>
<td>16.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Prenatal visits (#)</td>
<td>12 ± 3</td>
<td>12 ± 3</td>
<td>10 ± 3.1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>39.7 (38.7-40.7)</td>
<td>39.7 (38.7-40.7)</td>
<td>39 (38-40)</td>
<td>NS</td>
</tr>
<tr>
<td>Preterm delivery (%)</td>
<td>13</td>
<td>16</td>
<td>8.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cesarean section (%)</td>
<td>16</td>
<td>26</td>
<td>20.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3231 ± 525</td>
<td>3373 ± 578</td>
<td>3268 ± 513.3</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Continuous variables are reported as Mean ± SD or median (interquartile range) as appropriate.

** NS, not statistically significant.
† Visits refer to the number of routinely scheduled visits with obstetricians or midwives and excludes emergency, urgent care or unscheduled walk-in visits.

The rising PIH and MetS epidemic is largely a result of the rapidly developing affluence in the region which is often accompanied by the adoption of unhealthy diets and sedentary lifestyles [12-14, 23] and obesity [12, 24]. In the current study, relative affluence was associated with developing PIH where relatively affluent housing conditions (living in a villa) and middle-high salaries (10,000-14,999 QR/month) were more strongly associated with developing PIH. This is unlike other studies which tend to note that those in lower socio-economic status to be at higher risk of developing PIH [19, 21, 22]. In these studies this is attributed to lower standards of healthcare and this tends to be the case in higher economically developed nations.

It is unsurprising that obesity, being one of the main components of the MetS, is strongly associated with PIH in our study. Similarly, other studies have noted associations between obesity (BMI ≥ 30 kg/m²) and PIH with OR of 4.67 (95% confidence interval: 3.07-7.09) in an Dutch study [20]; OR 2.5 (95% CI 1.3-4.8) among Latin women in a US study [25] and OR 4.26 (95% CI 3.37-5.38) for those with a BMI of ≥ 40 kg/m² in another US study [26] and in Qatari’s studies [12-14]. Interestingly, in comparison to other published studies, our study has as of yet reported the highest OR odds (OR 10.0 95% CI 6.4-15.6) for associations between BMI ≥ 30 kg/m² and PIH.

In the current study, maternal age was also associated with PIH. In our study, risk of PIH linearly increased with each five years increase in age among pregnant women from 30 to 45 years of age. This finding is similar to what was reported in a Canadian study [27] which found that the odds increased by almost two-fold for incidence of PIH for women aged ≥ 35 years in comparison to those younger than 35 years. Another study conducted in Brazil found a five-fold increase (OR 5.218; 95% CI: 1.873-14.536) in odds of PIH among women aged over 30 years old in comparison to those younger than 30 years [28]; nonetheless age above 30 years was found to be protective against preeclampsia in this Brazilian study [28]. It is important to note, as reported in numerous international studies, that those below 20 years of age were at increased risk of preeclampsia [29]. We found increased risk among those who were above 30 years as they are more predisposed to the usual risks of hypertension that exists in the general population, namely obesity and type 2 DM [30-32].

In addition, there may be a genetic explanation for the high rates of PIH in Qatar. Our study demonstrated significantly higher rates of PIH among Qataris’ in comparison to other Arabs residing in Qatar. A recent study conducted in the USA comparing race and predisposition to gestational DM found Asians and Latinos, to have higher risk than Caucasians or African Americans [33]; The authors propose that Asians in general have a genetic predisposition to insulin resistance. It may well be the case that a similar genetic predisposition for PIH exists; nonetheless...
in the current study, family history of diabetes, hypertension, Down’s syndrome and consanguinity were not found to have significant associations with PIH at the multiple regression level.

In contrast to other studies [34, 35], having a previous abortion was not found to be protective, but rather increased the risk of developing PIH by 60% in our study. In addition, a more recent cohort study has found that a previous abortion was only protective for preeclampsia if they conceived with the same partner; this protective effect disappeared if they conceived with a different partner [36]. The discrepancy found between our study and those in the international literature may be due to the fact that our study included both gestational hypertension and preeclampsia patients as PIH rather than merely measuring for preeclampsia separately; indeed it has been suggested that these two conditions may have different etiologies, hence the difference in findings [37].

The fact that antenatal care was found to be protective indicates the importance of focusing efforts on this preventative factor. Indeed a study conducted in the US [38] found that Black women had worse outcomes than White women in relation to PIH as they were less likely to begin prenatal care in the first trimester of pregnancy and were less likely to receive adequate care. Similarly, a number of Dutch studies [7, 39] have noted that a number of maternal and neonatal deaths, which were a caused by hypertensive disorders of pregnancy, could have been prevented had more adequate care been taken by medical staff and had there been increased attendance to antenatal care. While the care provided in Qatar is universal, more public awareness campaigns are needed to encourage pregnant women to utilize these services early in their pregnancies.

Conclusion

In conclusion, Qatar has a high prevalence of PIH compared to both regional and global rates. Maternal age > 30, increased BMI, previous abortion, lack of antenatal care, and physical activity were found to be significantly associated with increased risk of PIH in Arab women and could be potentially modifiable risk factors.

Acknowledgement

This work was generously supported and funded by Qatar Foundation Grant No. UREP 08-046-3-010. We also would like to thank the Hamad Medical Corporation, Medical Research Committee (HMC Research Protocol No. 10145/10) and the Weill Cornell Medical College, Institutional Review Board (IRB# 2010-0022) for their ethical approval of this study.

References


The impact of socio-economic, lifestyle habits, and obesity in developing of pregnancy-induced hypertension in fast-growing etc.


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Arterial hypertension and female sexual dysfunction in postmenopausal women

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Summary

Purpose: To evaluate female sexual dysfunction in hypertensive postmenopausal women and the effects of antihypertensive therapy.

Materials and Methods: Female sexual dysfunction was assessed by the Female Sexual Function Index (FSFI) in three groups of postmenopausal patients: normotensive women (group A: 240 women), hypertensive women without therapy (group B: 220 women), hypertensive women on therapy (group C: 80 women). Results: The incidence of female sexual dysfunction was increased in group B compared to groups A and C. Healthy patients showed higher FSFI scores compared to hypertensive patients (groups B and C). Hypertensive-treated patients accounted for higher scores in all items compared to hypertensive patients without therapy. Conclusions: Essential hypertension significantly affects female sexual function. Physicians should recognize and properly manage FSD in hypertensive women.

Key words: Female sexual dysfunction; Hypertension; Postmenopause; Antihypertensive therapy.

Introduction

Female sexual dysfunction (FSD) is defined as a multifactorial problem including hypoactive sexual desire, arousal dysfunction, pain with intercourse (dyspareunia), tightening of the vaginal muscles to the point of discomfort and pain (vaginismus), and orgasmic dysfunction [1, 2]. Seventy-five percent of women experience sexual difficulties during their lifetime, and 50% of all married couples experience sexual dysfunction [2]; a marked increased incidence in postmenopause is associated with a decrease of estrogen levels resulting in progressive reduction of elasticity and lubrication of the vagina [3].

Until recently, most of these problems recognized psychological origins, but many medical conditions may be associated with increased risk of sexual dysfunction, including poor general health and cardiovascular disease [4-6]. This is probably most evident in essential hypertension which could theoretically affect most aspects of sexual functioning directly or indirectly through vascular problems, side-effects of medications, relationship problems, psychological disturbances, and other factors [7].

While a large body of evidence supports the impact of hypertension and of anti-hypertensive drugs on male sexual function [3], this topic remains underexplored in women, mainly in postmenopausal ones, although almost half of treated hypertensives are women. In this view, the aim of the present study was to evaluate the relationship between essential hypertension and FSD in a sample of postmenopausal women, and the effect of administration of anti-hypertensive therapy.

Materials and Methods

Five-hundred and forty women attending the outpatient menopausal clinic of the Second University of Naples were enrolled. Inclusion criteria were: spontaneous menopausal state lasting at least six months confirmed by plasma follicle-stimulating hormone (FSH) (> 40 IU/l) and estradiol (E2) (< 30 pg/ml) concentration, aged between 48 and 55 years and sexually active. Exclusion criteria were: vaginal dystrophy, diabetes or impaired glucose tolerance, uremia, multiple sclerosis, cancer, psychiatric disorders, cardiovascular diseases, urinary disorders, thyroid diseases, women with pelvic trauma, and alcohol and drugs assumption. Blood pressure (BP) was measured using a mercury sphygmomanometer and essential hypertension was defined as BP values equal to or greater than 140/90 mmHg, as by international classification of hypertension [8].

Three groups of patients were considered: normotensive patients (group A: 240 women) used as a controls, hypertensive patients without any antihypertensive treatment (group B: 220 women), patients on hypertensive therapy (group C: 80 women). FSD was defined as the persistent or recurrent decrease of sexual desire, the impairment of sexual act, as well as the presence of sexual impotence and pain after sexual contact. The Female Sexual Function Index (FSFI questionnaire) was used to evaluate FSD [9, 10]. FSFI questionnaire consists of 19 multiple-choice questions (and given a score from 0 to 5), used to investigate six areas: sexual desire (first two questions); excitement (from the third to the sixth question), lubrication (from the seventh to the tenth question), orgasm (from the 11th to the 13th question), satisfaction (from the 14th to the 16th question), and pain (from the 17th to the 19th question). FSD was defined as a score less than or equal to (≤) 26.

Results

This study showed that the FSD was reported in 48/240 (20%) of normotensive women, 84/220 (38%) of women with untreated hypertension, and 22/80 (27%) of women on anti-hypertensive treatment. FSD has higher preva-
Sexuality is a relevant determinant of well-being in postmenopause and is affected by many factors: while the effects of estrogen deficiency are well-studied, either directly to the genital mucous membrane dystrophy affecting dyspareunia, or indirectly through the negative influence on sexual desire, and orgasm, other factors that can negatively affect sexual function are much less investigated. The interest of the literature has been recently aimed at the cardiovascular risk factors involved in FSD. Some authors [5] showed that women with metabolic syndrome have a higher prevalence of FSD compared with healthy controls, although the association remains still not exactly explained. In a previous work the authors observed a relationship between FSD and obesity, demonstrating that the latter is able to influence various aspects of sexuality [11].

A rise in blood pressure causing endothelial dysfunction and impaired release of nitric oxide (NO) and catecholamines [12] may be associated with sexual dysfunction. In fact, it is necessary that vascular systems properly works in order for a woman to experience a normal arousal; in any case, eliminating cardiovascular risk factors decreases the risk of sexual dysfunction [3]. Indeed, the female genital arousal response is a neurovascular process characterized by genital engorgement, swelling, and lubrication. Disorders of arousal include decreased labial and clitoral sensation and engorgement, as well as lack of vaginal smooth muscle relaxation. It appears that NO plays a key role in clitoral smooth muscle relaxation, while its role in the vagina remains controversial. Functional adrenergic receptors are expressed both in the clitoris and in the vagina and mediate norepinephrine-induced genital smooth muscle contraction. Thus, it seems that the main mediators of male sexual function (NO and catecholamines) exert the same effects on female genital tissue as well. Moreover, angiotensin II seems to play a pivotal role in the structural and functional changes of the clitoris and vagina while blockade of the renin-angiotensin axis protects the genital tissue from these abnormalities [13].

While many studies were carried out on sexual dysfunction in hypertensive male subjects [14], less information is available regarding women and all the data are referred to premenopausal patients: sexual dysfunction is more prevalent in hypertensive women compared to normotensive women, hypertensive women experience decreased vaginal lubrication, less frequent orgasm, and more frequent pain compared to normotensive women, successful control of hypertension is related to lower prevalence of female sexual dysfunction, even if hypertension per se results in female sexual dysfunction rather than the antihypertensive therapy [1, 13, 15].

Recently, it has been hypothesized that postmenopausal estrogen deficiency contributes to enhance the alteration of NO, catecholamines, and angiotensin II occurring with hypertension, therefore the risk of FSD may be increased in postmenopausal hypertensive women. To the best of the authors’ knowledge, one study examined sexual function in postmenopausal women with heart disease and showed that anti-hypertensive medication was not a predictor of sexual problems [16]. The present study focused on a narrow category of postmenopausal women, and confirms the association between FSD and hypertension in agreement with the findings on premenopausal women. In addition, women treated with anti-hypertensives have less sexual dysfunction than hypertensive women without any pharmacological treatment to confirm the hypothesis that the reduction of blood pressure values is reflected in a lower degree of sexual dysfunction. As a consequence, adequate control of hypertension with medication not affecting sexual function can have a great impact on the quality of life of hypertensive patients [17].

The data in the present study need to be confirmed in larger case series, but they seem interesting in view of the high incidence of the metabolic syndrome in postmenopausal women, suggesting the need for a careful assessment of FSD that takes into account not only sociological parameters, but also new indicators, such as cardiovascular risk factors: because hypertension is a chronic disorder that requires patients to seek medical attention and visit their doctors regularly, such intense doctor-patient contact should normally provide an opportunity to address other health problems and therefore properly manage also FSD.
In conclusion, sexual function in post-menopausal women may be impaired by hypertension mainly when no pharmacological treatment is administered. Consequently, the assessment of sexual function should be considered for the management of hypertensive post-menopausal women.

References


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Gestational hypertension risk evaluation based on epidemiological, biochemical, and hemodynamic factors

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Summary

Purpose: Gestational hypertension (GH) is a serious health hazard for pregnant women and fetuses. The incidence of GH involves many epidemiological, biochemical, and hemodynamic factors. Methods: The current study investigated the GH risk under the influence of epidemiological, biochemical, and hemodynamic factors, and designed corresponding GH risk evaluation methods and apparatus. Results: The evaluation method has 74.15% sensitivity and 81.84% specificity. The ROC area under the curve is 0.841. The apparatus automatically imports epidemiological, biochemical, and hemodynamic factors, and then expresses the GH risk as numbers, bar codes, and colors through logic array analysis. Conclusion: The GH risk value can effectively give the risk level of GH. The GH risk barcode can improve the degree of automation of information storage, transmission, and identification in GH monitoring. The GH risk color can also improve the GH macro description.

Key words: Risk factors; Logistic regression; Logic switch array; Risk value; Risk bar code.

Introduction

Gestational hypertension (GH) is a serious health hazard for pregnant women and fetuses [1]. The incidence of GH involves many epidemiological factors. Pregnant women at a high gestational age (GA) [1, 2], are obese [1, 3, 4], have multiple pregnancies (MP) [5, 6], are positive for previous pregnancy-induced hypertension (P-PIH) [1], or have a spontaneous abortion history (SAH) [7, 8] have increased GH risk. The incidence of GH also involves many biochemical factors [3, 9]. Blood testing is a routine prenatal examination. Current studies report that pregnant women with GH have significantly different mean platelet volumes (MPV) [10-12], platelet count (PLT) [13, 14], and hematocrit (HCT) [15, 16] compared with normal pregnant women. In addition, the incidence of GH concerns many hemodynamic factors [17-22]. Pregnant women with GH have different hemodynamic changes compared with normal pregnant women, as detected through a non-invasive hemodynamic monitoring device produced by Beijing YES Medical Device Co., Ltd (China). Changes in the total peripheral resistance (TPR) and pulse waveform characteristic value (PWCV) are significantly correlated with GH risk [23-25].

The uterine artery resistance score (UARS) [26] and the nomogram method [27] are two GH risk evaluation techniques. Four indicators, namely, blood flow pulsatility indices, [28] resistance indices [29], systolic and diastolic blood flow velocity ratios (S/D), and early diastolic notch [30] of both sides of the maternal uterine artery are detected using color Doppler ultrasound at 24 to 25 weeks of gestation. UARS was established in the current study. The nomogram method entails setting the parity, previous preeclampsia, chronic hypertension, diastolic blood pressure, and proteinuria at certain values, with each value corresponding to the possible percentage of the corresponding risk. The final sum is then calculated as the GH risk.

UARS and the nomogram method have certain shortcomings. UARS only takes hemodynamic factors of GH into account and does not consider the epidemiological and biochemical factors. Therefore, the single score on the GH risk expression has some deviations. The nomogram method has more test parameters compared with UARS but does not consider the epidemiologic, biological, and hemodynamic factors in contributing to GH risk expression.

Therefore, the incidence of GH is affected by epidemiological, biochemical, and hemodynamic factors. However, GH risk evaluation methods are mostly one-sided, non-diverse, and noncomprehensive. In the current study, the GH results under the influence of epidemiological, biochemical, and hemodynamic factors were investigated, and GH risk evaluation methods and apparatus based on the foregoing factors were designed.

Methods and Results

GH risk evaluation method

In the present study, 751 pregnant women from the Beijing Obstetrics and Gynecology Hospital, a large maternity unit in China, were the research subjects. All pregnant women were requested to proceed with the first measurement. Those who had no concurrent obstetric or medical problems, such as cardiac disease, chronic hypertension, chronic illness, or long-
The final regression equation was as follows:

\[
\text{LogitP} = -3.055 + 2.274 \chi_{\text{P-PIH}} + 2.161 \chi_{\text{SP-MP}} + 1.625 \chi_{\text{PBMI}} + 1.455 \chi_{\text{SAH}} - 1.526 \chi_{\text{MPV}} - 0.676 \chi_{\text{PLT}} - 0.575 \chi_{\text{HCT}} + 2.283 \chi_{\text{TPR}} - 1.099 \chi_{\text{PWCV}}
\]

The chi-square test yielded the values \(\chi^2 = 311.296, p < 0.05\). The logistic regression equations were statistically significant. The probability of GH occurrence (PGH) was calculated using the formula below, and ROC curve analysis was performed. The ROC area was 0.841, the standard error was 0.016, and the 95% confidence intervals were 0.810-0.872.

\[
P_{\text{GH}} = \frac{e^{\text{LogitP}}}{1 + e^{\text{LogitP}}}
\]

A GH risk evaluation method based on epidemiological, biochemical, and hemodynamic factors was established. The epidemiological, biochemical, and hemodynamic factors of pregnant women were synthetically considered and logically quantified. The GH risk was expressed as the GH risk value, GH risk bar code, and GH risk color. The GH risk evaluation apparatus imported the logic switch array of the GH risk factor formation module, the hemodynamic factor import module, the logic switch array of GH risk factor formation module, the GH risk bar code generation module, the logic switch value regression module, the GH risk color generation module, and the GH risk display and output module.

As shown in Figure 1, the epidemiological, biochemical, and hemodynamic factors were imported from the epidemiological and biochemical factor import module and hemodynamic factor import module. The logical switch value of each factor was formatted using the logic switch array of the GH risk factor formation module; the GH risk bar code was generated by the GH risk bar code generation module; the GH risk value was computed by the logic switch value regression modules; and the GH risk color was generated by the GH risk color generation module. The final GH risk value, bar code, and color were exported on the display apparatus through the GH risk display and output module.

The epidemiological and biochemical factors of the pregnant women were exported into the epidemiological and biochemical factor import module, and the hemodynamic factors were imported into the hemodynamic factor import module.

The logic switch array of the GH risk factor formation module consists of logic switches for the epidemiological, biochemical, and hemodynamic factors. The logic switches for the epidemiological factors were P-PIH, SP-MP, PBMI, SAH, and GA alternately, with logic switch values of \(X_{\text{P-PIH}}, X_{\text{SP-MP}}, X_{\text{PBMI}}, X_{\text{SAH}},\) and \(X_{\text{GA}},\) respectively. The logic switches of the biochemical factors were MPV, PLT, and HCT alternately, with logic switch values of \(X_{\text{MPV}}, X_{\text{PLT}},\) and \(X_{\text{HCT}},\) respectively. The logic switches of the hemodynamic factors were TPR and PWCV, with logic switch values of \(X_{\text{TPR}}\) and \(X_{\text{PWCV}},\) respectively.

The logic switch value of each logic switch was 1 when the logic switch was turned on and 0 when the logic switch was turned off. The conditions for the logic switches and the corresponding logic values are shown in Table 1.
The GH risk bar code was generated from the GH risk bar code generation module in the order Xsp-Mp, Xp-bmi, Xp-mpv, Xs-ah, Xp-wcv, Xplt, Xga, Xhct, and XTPR, where Xsp, Xs, XTPR, and Xe are the logic switch values of the start and end tag switches, respectively. Xs was 1 and Xe was 0. Figure 2 shows a typical example of a GH risk bar code generated by the module, where the GH risk bar code was 110011100010 based on the example of a GH risk bar code generated by the module, where code generation module in the order

<table>
<thead>
<tr>
<th>Logic switch value</th>
<th>Quantitative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-PIH</td>
<td>Xp-PIH positive, Xp-PIH = 1; P-PIH negative, Xp-PIH = 0</td>
</tr>
<tr>
<td>SP-MP</td>
<td>Xsp-MP positive, Xsp-MP = 1; SP-MP negative, Xsp-MP = 0</td>
</tr>
<tr>
<td>PBMI</td>
<td>Xpbmi BMI ≥ 0.24 kg/cm², Xpbmi = 1; BMI &lt; 0.24 kg/cm², Xpbmi = 0</td>
</tr>
<tr>
<td>SAH</td>
<td>Xsa ah positive, Xsa ah = 1; SAH negative, Xsa ah = 0</td>
</tr>
<tr>
<td>GA</td>
<td>Xga GA ≥ 35, Xga = 1; GA &lt; 35, Xga = 0</td>
</tr>
<tr>
<td>MPV</td>
<td>Xmpv MPV positive, Xmpv = 1; MPV negative, Xmpv = 0</td>
</tr>
<tr>
<td>PLT</td>
<td>Xplt PLT positive, Xplt = 1; PLT negative, Xplt = 0</td>
</tr>
<tr>
<td>HCT</td>
<td>Xhct HCT positive, Xhct = 1; HCT negative, Xhct = 0</td>
</tr>
<tr>
<td>TPR</td>
<td>Xtp r TPR ≥ 1.2, Xtp r = 1; TPR &lt; 1.2, Xtp r = 0</td>
</tr>
<tr>
<td>PWCV</td>
<td>Xpwcv PWCV ≥ 0.4, Xpwcv = 1; PWCV &lt; 0.4, Xpwcv = 0</td>
</tr>
</tbody>
</table>

Table 2. — Multiple factors analysis results of GH.

<table>
<thead>
<tr>
<th>Logic switch value</th>
<th>p</th>
<th>OR</th>
<th>95% CI (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xp-PIH</td>
<td>0.078</td>
<td>9.718</td>
<td>0.776-121.687</td>
</tr>
<tr>
<td>Xsp-MP</td>
<td>0.000</td>
<td>8.679</td>
<td>2.827-26.644</td>
</tr>
<tr>
<td>Xpbmi</td>
<td>0.000</td>
<td>5.079</td>
<td>3.253-7.931</td>
</tr>
<tr>
<td>Xsa ah</td>
<td>0.001</td>
<td>4.283</td>
<td>1.771-10.355</td>
</tr>
<tr>
<td>Xga</td>
<td>0.003</td>
<td>1.693</td>
<td>1.203-2.384</td>
</tr>
<tr>
<td>Xmpv</td>
<td>0.000</td>
<td>4.602</td>
<td>3.043-6.985</td>
</tr>
<tr>
<td>Xplt</td>
<td>0.089</td>
<td>1.954</td>
<td>0.903-4.226</td>
</tr>
<tr>
<td>Xhct</td>
<td>0.053</td>
<td>0.563</td>
<td>0.315-1.007</td>
</tr>
<tr>
<td>Xtp r</td>
<td>0.000</td>
<td>9.809</td>
<td>5.297-18.163</td>
</tr>
<tr>
<td>Xpwcv</td>
<td>0.001</td>
<td>3.002</td>
<td>1.554-5.800</td>
</tr>
</tbody>
</table>

The GH risk bar code was generated from the GH risk bar code generation module in the order Xp-PIH, Xsp-MP, Xs-ah, Xp-wcv, Xplt, Xga, Xhct, and XTPR, and Xs, Xe and Xp are the logic switch values of the start, middle, and end tag switches, respectively. Xs was 1 and Xp was 0. Figure 2 shows a typical example of a GH risk bar code generated by the module, where the GH risk bar code was 110011100010 based on the example of a GH risk bar code generated by the module, where code generation module in the order

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The final GH risk value, bar code, and color were exported on the display apparatus through the GH risk display and output module.

### Discussion

Gestational age (GA) above 30 years is a risk factor for preeclampsia superimposed on chronic hypertension and a protective factor against preeclampsia [1]. Thadhani et al. [32] found that obese pregnant women have a more increased risk of GH than non-obese women. Compared with women with a pregravid BMI of 21-22.9 kg/m², the relative risk of GH was 1.6 for women with BMI of 23-24.9 kg/m², 2.0 for BMI 25-29.9 kg/m², and 2.6 for BMI over 30 kg/m². Multiple pregnancies are another risk factor for GH. The logistic regression shows that twin pregnancy carries a relative risk of 3.5 [33]. Other studies showed that a positive family history of hypertension, hypercholesterolemia, chronic hypertension, and gestational diabetes are closely related to increased risk of GH [34-36].

Pregnant women with TPR greater than or equal to 1.2 and with PWCV greater than or equal to 0.4 have significantly higher GH incidence. The results were 76.9% sen-
sitivity and 74.7% specificity [24]. Yu et al. [37] developed a predictive model for preeclampsia and found that the combination of uterine artery Doppler ultrasound and maternal factors provides the best estimate of risk. Gomez et al. [38] analyzed the uterine artery Doppler at 11-14 weeks of gestation to screen for hypertensive disorders and associated complications in an unselected population. They produced a scoring system with a sensitivity of 23.9% and a specificity of 93.8% to screen all complications of a mean PI > 95th percentile, which was found in 53/999 pregnancies. In the screening for preeclampsia, the detection rate for a 5% false-positive rate was 14.1% for PAPP-A, 54.7% for uterine artery mean PI, and 62.1% for a combination of PAPP-A and uterine artery mean PI [39]. Lee et al. [40] integrated a multifactorial model based on mid-trimester beta-hCG levels for the prediction of severe preeclampsia, with a sensitivity of 70% and a specificity of 71%. The mean MSuE [3] levels in patients with early onset were significantly lower than in patients with late-onset severe preeclampsia. High MSAFP and hCG and low MSuE [3] may be significant markers of early- rather than late-onset severe preeclampsia [41].

UARS has four indicators, namely, the blood flow pulsatility indices, resistance indices, systolic and diastolic blood flow velocity ratios (S/D), and early diastolic notch of both sides of the maternal uterine artery. The UARS for the prediction of PIH also appeared statistically significant ($p < 0.01$), with its optimal cutoff level ≥ 4 scores. The value of UARS for the prediction of PIH was much higher than that of other single parameters, with 50% sensitivity and 98.9% specificity [26]. Deis et al. [27] created a nomogram for the individual prediction of preeclampsia based on multivariate analysis, nulliparity, previous preeclampsia, diastolic blood pressure, biparietal diameter, and umbilical artery Doppler resistance index which were introduced into a nomogram with an area under the ROC curve = 0.73.

Therefore, GH risk evaluation methods and apparatus were established by considering the high risk factors of GH, including epidemiological, biochemical, and hemodynamic factors. The risk factors were quantified, valued, and demonstrated in the evaluation apparatus. The sensitivity and specificity were 74.15% and 81.84%. The ROC area under the curve was 0.841. The combination of epidemiological, biochemical, and hemodynamic factors in evaluating the risk of GH produced better results than the single-factor evaluation method. The evaluation methods and apparatus may determine the possible occurrence of GH for early intervention and for reduction of maternal and child hazards.

Conclusion

GH risk evaluation methods and apparatus based on epidemiological, biochemical, and hemodynamic factors were proposed. The GH risk evaluation methods and apparatus automatically imported epidemiological, biochemical, and hemodynamic factors from the case manage ment computer, monitored the GH noninvasive hemodynamic factors, and expressed the GH risk as values, bar codes, and colors through logic array analysis. The GH risk values, including $P_{GU}$ and $f_{GH}$, can effectively yield the GH risk level, which is important for early prediction, early detection, and early intervention of GH and for improving the quality of perinatal care. The GH risk bar code can improve the degree of automation of data storage, transmission, and identification in GH monitoring. The GH risk color can improve the macro description of the GH risk.

Acknowledgments

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References

Gestational hypertension risk evaluation based on epidemiological, biochemical, and hemodynamic factors


Relevance of anti-Müllerian hormone on in vitro fertilization outcome

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Department of Obstetrics and Gynecology, Istanbul University School of Medicine, Istanbul (Turkey)

Summary

Purpose: The aim of this study was to investigate the relevance of serum and follicular anti-Müllerian hormone (AMH) concentrations on ovarian reserve and clinical pregnancy. Materials and Methods: Thirty patients were prospectively included in this study. Serum AMH levels were quantitatively measured on the follicle aspiration day. Retrieving less than five oocytes was defined as poor response. Eleven days after embryo transfer, beta-human chorionic gonadotropin (β-hCG) level in the blood was measured. Two weeks after the β-hCG test, a clinical pregnancy was confirmed by transvaginal ultrasound (TVUS). Results: There was a statistically significant correlation between serum AMH and number of retrieved oocytes (p = 0.024). There was a correlation between the number of retrieved oocytes and baseline antral follicle count (AFC), between ovarian reserve and baseline follicle-stimulating hormone (FSH), and between ovarian reserve and serum AMH (p < 0.05). Serum AMH cut-off value for the normal ovarian reserve was calculated as 0.37 ng/ml (sensitivity 71.43%, specificity 66.67%, positive prediction 83.33%, negative prediction 50%). Conclusion: Increasing use of serum AMH will be of considerable benefit. Consequently, the observed positive correlation between serum AMH and ovarian reserve will require larger sampling to refine the role of AMH.

Key words: Anti-Müllerian hormone; Follicular anti-Müllerian hormone; In vitro fertilization; Serum anti-Müllerian hormone.

Introduction

Socio-economic changes in several societies have an impact on couples’ desires to have children. Consequently, in the last decade, more couples postpone their plans to have children. On the other hand, it has been well-established that with an increasing age, female fertility decreases. This has been clearly demonstrated by the age-dependent success rates of assisted reproductive technology (ART) therapy [1]. Changes in ovarian reserve, which are defined as the number and quality of the follicles and oocytes in the ovaries at a given age, lead to age-related female infertility [2]. For ovarian reserve testing prior to ART, the age of the patient remains the first line of choice as a predictor. However, a test that can provide accurate information on a patient’s ovarian reserve would be of immense help to any clinician.

Recently, a role for anti-Müllerian hormone (AMH) in ovarian function has become apparent by the aid of animal studies [3]. The release of AMH from ovarian granulosa cells paves the way to measurable serum levels, which are proportional to the number of follicles in the ovaries. Hence, AMH can be considered as one of the markers for ovarian aging, since the number of follicles decreases with age [4].

The aim of this study was to investigate the relevance of serum and follicular anti-Müllerian hormone (AMH) concentrations on ovarian reserve and clinical pregnancy, which are important success parameters in an ART therapy like in vitro fertilization (IVF).

Materials and Methods

Thirty IVF patients in the Infertility Clinic of Istanbul University School of Medicine were prospectively included within this study. Inclusion criteria were as follows: 18-45 years of age, no history of an endocrine disorder of the thyroid, adrenals, etc., and no history of an ovary-related surgery. Approval of the ethics committee and informed consent from all participants were obtained prior to the treatment.

Patients were assessed by day-3 hormone profile follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P), prolactin (PRL), thyroid-stimulating hormone (TSH), and hysterosalpingography. Transvaginal ultrasonography (TVUS) was performed on the second or third day of the menstrual cycle and the size of the uterus and of the ovaries, follicle count and diameter measurements were recorded. Serum AMH levels were quantitatively measured on the follicle aspiration day in the microbiology laboratory of Istanbul University School of Medicine by enzyme-linked immunosorbent assay (ELISA) and applied uniformly for all patient samples. Intra- and interassay coefficients of variation with serum controlled samples were 4.6% and 5.2%, respectively. The type of gonadotropin releasing hormone (GnRH) agonist long protocol or GnRH antagonist protocol for ovarian stimulation was determined by the patient’s doctor, based on her age, and the status of ovarian reserve markers. The number of follicles and endometrial thickness were recorded at each follow-up.

Follicular fluid sample of the leading follicle collected during oocyte retrieval (OCT) was centrifuged at 3,000 cycles/min and stored at -80°C for follicular AMH measurement. All follicles that were ≥ 14 mm in size were aspirated. The number of retrieved oocytes was recorded. Retrieving less than five oocytes was defined as a poor response. Three days after retrieval, one to three embryos (grade-1) were transferred to the uterine cavity depending on the age of the patient. Eleven days after embryo transfer, beta-chorionic gonadotropin (β-hCG) level in the blood was measured. If β-hCG level was > 5 mIU/ml in either measurement, it was considered positive β-hCG and patients with such levels were regarded as biochemically pregnant. Two

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weeks after the β-hCG test, clinical pregnancy was confirmed by TVUS.

All statistical calculations were performed using the NCSS 2007 and PASS 2008 (NCSS, Kaysville, UT, USA). Data are presented as mean ± SD. Parametric variables were evaluated by Student's t-test, while non-parametric variables were evaluated by the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analysis was used to determine cut-off values for those parameters significantly associated with clinical pregnancy and ovarian reserve. Statistical significance was defined as p < 0.05.

Results

Demographic and cycle characteristics of the study population are presented in Table 1. There was no correlation between serum AMH and age, baseline FSH or baseline antral follicle count (AFC) (p > 0.05). On the other hand, there was a statistically significant correlation between serum AMH and number of retrieved oocytes (p = 0.024). There was no correlation between follicular AMH and age, number of retrieved oocytes, baseline FSH or baseline AFC (p > 0.05). There was a negative correlation between baseline FSH and number of retrieved oocytes (p < 0.01) and also between baseline FSH and baseline AFC (p < 0.05). There was a correlation between the number of retrieved oocytes and baseline AFC (p < 0.05). There was no correlation between age and baseline FSH, number of retrieved oocytes, or baseline AFC (p > 0.05). There was no correlation between clinical pregnancy and serum/follicular AMH, age, baseline AFC, baseline FSH, baseline E2, period of infertility, number of metaphase II oocytes, number of retrieved oocytes, or endometrial thickness (p > 0.05).

The area under the ROC curve for predicting clinical pregnancy was larger for follicular AMH (0.630; 95% confidence interval CI: 0.419-0.841; p = 0.28) than for serum AMH (0.594; 95% CI: 0.344-0.842; p = 0.43), age (0.446; 95% CI: 0.221-0.670; p = 0.65) and FSH (0.383; 95% CI: 0.143-0.623; p = 0.33), but not statistically significant.

There was no correlation between ovarian reserve and follicular AMH, age, baseline AFC, baseline E2, or period of infertility (p > 0.05). There was a correlation between ovarian reserve and baseline FSH (p < 0.05). Baseline FSH of patients with a normal ovarian reserve was significantly lower than patients with a poor ovarian reserve (5.95 ± 1.85 vs 9.02 ± 4.52). There was a correlation between ovarian reserve and serum AMH (p < 0.05). Serum AMH concentrations of patients with a normal ovarian reserve were significantly higher than patients with a poor ovarian reserve (0.73 ± 0.63 vs 0.28 ± 0.19).

The area under the ROC curve for poor ovarian responders was larger for serum AMH (0.775; 95% CI: 0.604-0.946; p = 0.19) than for AFC (0.714; 95% CI: 0.518-0.911; p = 0.067) and follicular AMH (0.545; 95% CI: 0.313-0.777; p = 0.700) and statistically significant. Due to the correlation between serum AMH and ovarian reserve, ROC curve analysis was used to determine the cut-off value for serum AMH significantly associated with ovarian reserve. Serum AMH cut-off value for a normal ovarian reserve (five oocytes or more) was calculated as 0.37 ng/ml (sensitivity 71.43%, specificity 66.67%, positive prediction 83.33%, negative prediction 50%) (Table 2).

Table 1. — Patient characteristics (p = 30).

<table>
<thead>
<tr>
<th>Serum AMH (ng/ml)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive prediction (%)</th>
<th>Negative prediction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>85.71</td>
<td>44.44</td>
<td>78.26</td>
<td>57.14</td>
</tr>
<tr>
<td>0.30</td>
<td>76.19</td>
<td>55.56</td>
<td>80.00</td>
<td>50.00</td>
</tr>
<tr>
<td>0.37</td>
<td>71.43</td>
<td>66.67</td>
<td>83.33</td>
<td>50.00</td>
</tr>
<tr>
<td>0.40</td>
<td>61.90</td>
<td>77.78</td>
<td>86.67</td>
<td>46.67</td>
</tr>
<tr>
<td>0.45</td>
<td>52.38</td>
<td>77.78</td>
<td>84.62</td>
<td>41.18</td>
</tr>
<tr>
<td>0.50</td>
<td>47.62</td>
<td>88.89</td>
<td>90.91</td>
<td>42.11</td>
</tr>
<tr>
<td>0.60</td>
<td>47.62</td>
<td>100.00</td>
<td>100.00</td>
<td>45.00</td>
</tr>
<tr>
<td>0.70</td>
<td>38.10</td>
<td>100.00</td>
<td>100.00</td>
<td>40.91</td>
</tr>
</tbody>
</table>

Table 2. — Serum AMH cut-off values.

Table 3. — Patient characteristics as a function of serum AMH.

Discussion

The correct assessment of ovarian reserve is crucial for a successful IVF outcome. In this study, the authors sought to determine whether serum or follicular AMH would have any use in predicting ovarian reserve and subsequently, clinical pregnancy rates.

Mattukrisha et al. found a correlation between serum AMH and number of retrieved oocytes in a study consisting of 69 patients (p < 0.001) [5]. Buyuk et al. carried out a somewhat similar study and found that serum AMH levels strongly correlate with the number of retrieved oocytes.
oocytes as well (p < 0.0001) [6]. The findings in the presented study yielded a correlation between serum AMH and the number of retrieved oocytes as well (p = 0.024). Sills et al. [7], in a study of 79 patients, found a moderate positive correlation between serum AMH and the number of metaphase II oocytes; the present study also revealed a correlation that was not statistically significant (p = 0.078). Riggs et al. also stated that serum AMH values correlated the best with the number of retrieved oocytes (p = 0.001) relative to age (p < 0.01) and FSH (p < 0.01) [8]. In the present study, there was no correlation between serum AMH and age (p = 0.929) or baseline FSH (p = 0.111).

The authors demonstrated a clinical pregnancy rate of 27% while none of the parameters achieved a statistical significance in predicting the clinical outcome. In contrast, Wu et al. conducted a study that included 60 patients and found a statistically significant correlation between clinical pregnancy outcome and day-3 AMH (p < 0.05) [9]. On the other hand, Buyuk et al. found a correlation between serum AMH and the clinical outcome that was not statistically significant (p = 0.1) [6]. Sills et al. also indicated a higher serum AMH level in patients who attained a clinical pregnancy, but the difference was not significant (p = 0.14) [7]. Additionally, a meta-analysis by Broekmans et al. revealed that the accuracy of the current ovarian reserve tests, including serum AMH, for predicting the occurrence of pregnancy, is very limited [10].

Poor ovarian reserve had a strong correlation with serum AMH in the present study, which was seen in 30% of the sample size (p = 0.017). Van Rooij et al. defined “a poor response” as retrieving less than four oocytes and found it strongly correlated with serum AMH as well (p < 0.01) [11]. In a recent study by Tolikas et al., “a poor response” was again defined as retrieving less than four oocytes [12]. These researchers presented significant differences between poor and normal responders regarding FSH (p = 0.019) and serum AMH (p = 0.002), but not for follicular AMH (p = 0.183). The present study also demonstrated significant differences between poor and normal responders regarding FSH (p = 0.01), while no difference was found regarding follicular AMH (p = 0.722).

The strong correlation between serum AMH and ovarian reserve led the authors to determine the cut-off value for serum AMH by using ROC curve. Serum AMH cut-off value for the normal ovarian reserve (five oocytes or more) was calculated as 0.37 ng/ml. This data suggests that women with increased serum AMH concentrations above 0.37 ng/ml may be regarded as a better-prognosis group during IVF cycles than women with a serum AMH below 0.37 ng/ml. There have been several studies that attempted to determine a cut-off serum AMH value for a normal ovarian reserve, albeit with varying results (Table 4). In more recent ones, Jayaprakasan et al. [13] found 0.99 ng/ml as the optimum serum AMH cut-off value, Buyuk et al. [6] argued that women who had a serum AMH level of 0.6 ng/ml or higher had a better ovarian reserve, while Tolikas et al. presented a higher serum AMH cut-off value of 2.74 ng/ml [12]. In a review, Broer et al. concluded that serum AMH was able to predict ovarian reserve but could not predict pregnancy after ART treatment [14]. Therefore, this review’s authors advocated the determination of a low serum AMH cut-off value due to the importance of statistical specificity in poor ovarian reserve.

The findings in this study indicated that after serum AMH, baseline AFC was the most effective parameter in predicting ovarian reserve, yet it was not statistically significant (p = 0.067). On the other hand, the patients with serum AMH ≤ 0.37 ng/ml had a significantly lower baseline AFC (p < 0.05). Both Jayaprakasan et al. [13] and Tolikas et al. [12] concluded that a baseline AFC and serum AMH are significant predictors of poor ovarian reserve to ovarian stimulation during IVF, while Nardo et al. concluded that serum AMH was superior to AFC in its ability to predict poor response [15].

In conclusion, serum AMH appears to represent a compelling tool for the assessment of ovarian reserve, yet it yields a non-significant predictive value for clinical pregnancy. While bearing the caveats, it is still clear that the increasing use of serum AMH will be of considerable benefit in reproductive medicine. Consequently, the observed positive correlation between serum AMH and ovarian reserve will require larger sampling to refine the role of AMH in IVF strategies.

References
Relevance of anti-Müllerian hormone on in vitro fertilization outcome

69


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Doppler parameters of maternal renal blood flow in normal pregnancy

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Summary
The purpose of this investigation was to evaluate changes in maternal renal arterial blood flow during pregnancy. Materials and Methods: The study included 40 non-pregnant, 200 pregnant, and 30 women after delivery. The authors measured pulsatility index (Pi) and resistance index (Ri) in the right and left renal arteries in the hilus. The authors compared the values between non-pregnant and women during first, second, and third trimester and post-partum period and tested correlation with gestational age. Results: The authors did not find a statistical difference in Pi and Ri between the right and left kidneys. There was no difference in Pi and Ri in pregnancy trimester compared to the non-pregnant state. There was no correlation between the values of Pi and Ri and gestational weeks. Conclusion: During pregnancy there are no changes in the values of maternal renal Pi and renal Ri. Unchanged total vascular resistance may result from physiological changes of the glomerular filtration rate. Key words: Kidney; Maternal; Renal artery; Doppler; Pregnancy.

Introduction
During pregnancy, changes in maternal circulation occur that are the result of intense arteriolar vasodilatation and are essential for successful pregnancy outcome [1]. The application of Doppler ultrasound for the evaluation of maternal circulatory changes could be useful in the intensive follow up of high-risk pregnancies with preeclampsia. Along with uterine circulation, other vascular beds that may be of interest during pregnancy are those also affected with preeclampsia. The kidney is usually affected in severe preeclampsia and the most severe condition may result in acute renal failure [2]. Early detection of renal affection during preeclampsia and follow up of renal diseases may include Doppler evaluation of renal circulation. However, the renal circulation must be evaluated in normal pregnancy, so that comparison with normal blood flow can be made in potentially hazardous conditions. Until now there are many studies concerning renal blood flow in normal pregnancy and in pregnancy hypertension [3-7]. These studies were usually performed with a limited number of patients, and a study evaluating substantial number of patients may be of importance. The purpose of this investigation was to evaluate the presence of changes in Doppler resistance indices in maternal arterial renal blood flow depending on gestational age by comparing the values in pregnancy with the values before pregnancy and after delivery.

Materials and Methods
This clinical study included 200 healthy pregnant women with a gestational age between six and 40 weeks and 40 healthy non-pregnant women. There was no previous history of any chronic disease in all the cases (essential hypertension, diabetes mellitus, liver, renal, or cardiac disease). All the pregnancies were single, with no presence of gestational hypertension or preeclampsia. Institutional approval for the study was granted by the ethical committee and each patient signed informed consent form for the participation in this study.

The authors also measured renal blood flow by assessing the renal artery (RA) at the renal hilus before it branches into the interlobar arteries by pulsed Doppler ultrasound (Voluson Expert Pro) with a 3.5 MHz convex transducer. All the measurements were recorded after at least six hours of fasting and ten minutes rest. The subjects were in the left and right lateral positions and instructed to suspend respiration during measurements. The kidneys were first visualized in the B-mode image. In all the evaluated subjects, there were no changes in the renal position, size, contour, and parenchymal structure [8]. After obtaining an optimum B-mode view, color flow was activated and blood flow was measured under the beam angle under 30°. The authors measured the pulsatility index (Pi) and the resistance (Ri) index, calculated by original computer software, first in right and then in left RA (Figure 1). At least three similar sequential Doppler waveforms were measured. A mean value was calculated from the resistance indices for each kidney and mean intrarenal Pi and Ri were also calculated [8, 9].

Pregnant women (200) were divided according to pregnancy trimester: 30 women in the first trimester group; 85 in the second trimester group; and 85 in the third trimester group. From the third trimester group, 30 women after delivery were evaluated.

The authors compared the values between the subjects who were not pregnant and pregnant women during the first, second,
and third trimesters of pregnancy and those evaluated at postpartum as well. Correlation of Pi and Ri with gestational age were also tested and correlation curves were made for both parameters. A comparison was then made between the values of the right and left kidneys and between intrarenal values in all the groups.

The statistical analysis was performed with the SPSS program version 10 (SPSS INC, Chicago, IL) using chi-square test, one way ANOVA, followed by post-hoc, and two-tailed Pearson and Spearman correlation. The difference was considered to be significant if \( p < 0.05 \).

All the measurements were performed by two of the most experienced investigators (VM and MD) and calculated intraobserver and interobserver reproducibility by using intraclass correlation coefficient – RI. The agreement was considered acceptable from a clinical point of view when RI value was \( \geq 0.60 \).

### Results

There were no significant differences between the groups in the maternal age (\( t = 0.364; p > 0.05 \)) and parity (Chi^2 = 4.486; \( p > 0.05 \)). Both systolic (\( F = 1.989; p < 0.05 \)) and diastolic (\( F = 2.704, p < 0.01 \)) blood pressure were significantly lower in third trimester group (Table 1).

The authors did not find significant difference in the values of Pi of the right (\( F = 1.4, p > 0.05 \)) and left kidneys (\( F = 0.327, p > 0.05 \)), nor the mean intrarenal Pi (\( F = 0.62, p > 0.05 \)) and Ri of the right (\( F = 0.603, p > 0.05 \)) and left kidneys (\( F = 1.578, p > 0.05 \)), nor the mean intrarenal Ri (\( F = 1.415, p > 0.05 \)) among the pregnant, non-pregnant and postpartal groups. There were no difference between the values of Pi (\( t = 0.948; p > 0.05 \)) and Ri (\( t = 1.389; p > 0.05 \)) in the right and left sides, Table 2.

The authors did not observe the presence of correlation between mean values of evaluated parameters and parity (\( Pi, r = -0.181, p > 0.05 \); \( Ri, r = -0.230, p > 0.05 \)), maternal age (\( Pi, r = -0.054, p > 0.05 \); \( Ri, r = 0.043, p > 0.05 \)), nor the values of systolic (\( Pi, r = -0.098, p > 0.05 \); \( Ri, r = -0.086, p > 0.05 \)) and diastolic blood pressure (\( Pi, r = -0.059, p > 0.05 \); \( Ri, r = -0.184, p > 0.05 \)).

Furthermore the authors did not observe correlation between the values of mean intrarenal Pi (\( r = -0.096, p > 0.05 \)) and Ri (\( r = -0.113, p > 0.05 \)) with the gestational age (Figure 2).

Interobserver variability was good for both Pi and Ri (Pi – 0.69; Ri – 0.68). Intraobserver variability was also good for both Pi and Ri (Pi – 0.62; Ri – 0.63). Thus, both interobserver and intraobserver reproducibility of blood flow measurements were clinically acceptable in this study.

### Discussion

Doppler examination of the renal circulation has proved to be useful in assessing kidney failure. Factors that must be taken into account when assessing renal circulation are: age, acute renal failure, obstruction of renal pelvis, extrarenal compression, low diastolic blood pressure, bradycardia, and interstitial scarring. Comparable values are obtained only when arteries of equal order are sampled [6, 10].

The authors considered justified the choice of assessing RA in the hilus, as hiliar blood flow reflects downstream flow, and Ri is an index of the kidney's peripheral arterial resistance. Although intrarenal arteries are significant in the evaluation of parenchymal blood flow, the authors chose the RA as there is no significant variation in vascular resistance among different branches of the renal circulation [10]. Furthermore, the authors did not experience any technical problems during ultrasound surveys, although the subjects were pregnant, the majority being in the second and third trimester. The physiological pyeloclyses were discarded, as they do not influence renal blood flow [11].

The authors measured renal blood flow by using Ri. In all other studies, Ri were measured [3, 5–7, 11–17], but also peak systolic and end-diastolic velocities, acceleration time, and systolic acceleration [6, 17]. Although the measurement of different parameters is certainly superior to measurement of only one parameter, the authors chose the Ri because it is more reliable as Doppler beam angle may vary in different measurements [9, 10].

Furthermore, the authors did not find any difference in Pi and Ri between the right and the left kidneys, and therefore decided to introduce mean intrarenal parameters. In

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### Table 1: Maternal age, parity, and systolic and diastolic arterial blood pressure in the groups of non-pregnant, pregnant women during first, second, and third trimesters, and after delivery.

<table>
<thead>
<tr>
<th>Group</th>
<th>Maternal age (years)</th>
<th>Parity - mulipara</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 40</td>
<td>n = 30</td>
<td>n = 85</td>
<td>n = 85</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>29.90 ± 0.156</td>
<td>27.95 ± 0.136</td>
<td>27.51 ± 0.140</td>
<td>27.98 ± 0.156</td>
</tr>
<tr>
<td>First trimester</td>
<td>30.90 ± 0.156</td>
<td>29.05 ± 0.136</td>
<td>28.95 ± 0.140</td>
<td>29.23 ± 0.156</td>
</tr>
<tr>
<td>Second trimester</td>
<td>31.00 ± 0.156</td>
<td>29.10 ± 0.136</td>
<td>29.10 ± 0.140</td>
<td>29.40 ± 0.156</td>
</tr>
<tr>
<td>Third trimester</td>
<td>31.10 ± 0.156</td>
<td>29.20 ± 0.136</td>
<td>29.20 ± 0.140</td>
<td>29.50 ± 0.156</td>
</tr>
<tr>
<td>After delivery</td>
<td>31.20 ± 0.156</td>
<td>29.30 ± 0.136</td>
<td>29.30 ± 0.140</td>
<td>29.60 ± 0.156</td>
</tr>
</tbody>
</table>

* - p < 0.05; § - p < 0.01

### Table 2: The renal artery pulsatility and resistance indices in the right and left kidneys and mean intrarenal values in the groups of non-pregnant and pregnant women during first, second, and third trimesters and after delivery.

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Pi</th>
<th>Right Ri</th>
<th>Left Pi</th>
<th>Left Ri</th>
<th>Mean intrarenal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>1.031</td>
<td>1.026</td>
<td>1.028</td>
<td>0.605</td>
<td>0.594 ± 0.601</td>
</tr>
<tr>
<td>First trimester</td>
<td>1.049</td>
<td>1.031</td>
<td>1.037</td>
<td>0.622</td>
<td>0.615 ± 0.619</td>
</tr>
<tr>
<td>Second trimester</td>
<td>1.048</td>
<td>1.032</td>
<td>1.040</td>
<td>0.623</td>
<td>0.622 ± 0.624</td>
</tr>
<tr>
<td>Third trimester</td>
<td>1.000</td>
<td>1.011</td>
<td>1.001</td>
<td>0.616</td>
<td>0.607 ± 0.609</td>
</tr>
<tr>
<td>After delivery</td>
<td>1.048</td>
<td>1.031</td>
<td>1.040</td>
<td>0.611</td>
<td>0.608 ± 0.610</td>
</tr>
</tbody>
</table>

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<tr>
<th>Group</th>
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<tr>
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</tr>
</tbody>
</table>

* - p < 0.05; § - p < 0.01
healthy subjects, the \( R_i \) values will show only minimal difference within one kidney and between kidneys [8-10]. Previous studies confirmed that there was no difference in \( R_i \) between the right and left sides [11-14] and mean intrarenal parameters were also used by other authors [11, 13]. The authors considered justified the calculation of mean values, as only healthy subjects with normal renal function were evaluated and the conditions that could change renal blood flow would potentially influence both kidneys. Evaluation of renal circulation may be done by measuring intrarenal values or only by measuring the right side which may be preferred for technical convenience.

The authors did not find correlation between the RA, \( R_i \) with the values of systolic and diastolic blood pressure, which is in accordance with the results of previous studies [15]. The absence of correlation between blood pressure and \( R_i \) may be explained by the fact that the values of blood pressure do not influence the vessel impedance.

The results in the present study show that there is no significant difference between \( P_i \) and \( R_i \) values during pregnancy and the values of non-pregnant women and women after delivery. These results are similar to the results of many previous studies [3, 7, 12-14, 16]. The majority of these studies evaluated a small number of participants, mostly in late second and third trimesters, and they mixed normal and hypertensive pregnancies, while this study evaluated 200 normal pregnancies [3, 7, 13-14, 16]. The only study that assessed a significant number of subjects (\( n = 338 \)) included only women at 21-24 weeks gestation [12]. The current study is instead the first that includes pregnant women in all gestational weeks including women after delivery.

Inter- and intra-observer reproducibility was good for both \( P_i \) and \( R_i \). This is contributed by the fact that the two most experienced investigators performed all the exams. Other studies emphasize that operator experience is the most important in obtaining reliable results [17, 18].

Concerning the given results, a question could be raised, why the values of \( R_i \) are unchanged during pregnancy. In all the other evaluated maternal circulations, beginning from uterine, decreased \( R_i \) that are the result of intense arteriolar vasodilatation are observed and they are essential for the successful pregnancy outcome [19]. Along with the uterus, the kidney is the organ with the highest blood flow during pregnancy, as plasma volume is increased by 60%-80% and significant decrease of \( R_i \) throughout the gestation could be expected [1]. The absence of these changes may be explained by normal increase of glomerular filtration rate (GFR) which increases up to 50% from the sixth gestational week [1, 2]. GFR depends upon glomerular capillary pressure which is the product of the glomerular blood flow and glomerular resistance. Glomerular blood flow reflects renal blood flow and depends upon the afferent arteriole (pre-glomerular) tone that depends upon arterial blood pressure. Glomerular resistance depends upon efferent arteriolar tone. An increase of glomerular capillary pressure and GFR is achieved by the vasodilatation of afferent and moderate vasoconstriction of efferent arterioles vessels, while severe vasoconstriction of efferent vessels causes reduced glomerular filtration [20]. Therefore, unchanged total vascular resistance in normal pregnancy might be the result of physiological hyperfiltration especially during the first and second trimesters. Decreased tonus of the afferent and increased tonus of the efferent arterioles may be under the influence of hormones during pregnancy, predominantly progesterone, along with the influence of local factors.

The authors may conclude that during pregnancy, maternal renal circulation undergoes no significant changes in relation to the values of renal pulsatility and resistance indices. Unchanged total vascular resistance could be the increased tonus of efferent arterioles. Evaluation of renal circulation may be expressed in intrarenal mean pulsatility and resistance indices, or by Doppler measuring only in the right kidney.

References


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Comparison of transvaginal 3D sonohysterography with outpatient hysteroscopy in the evaluation of abnormal uterine bleeding

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Summary

Objective: To compare transvaginal three-dimensional sonohysterography (3D SHSG) and outpatient hysteroscopy with regards to diagnostic accuracy, procedure time, and patient discomfort with a prospective randomized controlled cohort study in a teaching hospital in London. The study included a population group of 49 women with abnormal uterine bleeding from varied ethnic backgrounds, of which 44 completed the study. Subjects with pregnancies, pelvic infections, large uteruses, suspicious or diagnosed pelvic malignancies, and who did not meet the criteria for day surgery, were excluded. Materials and Methods: Patients were randomized into two groups: group 1 had hysteroscopy followed by SHSG while group 2 had SHSG followed by hysteroscopy. Diagnostic accuracy, procedure time, and patient discomfort of SHSG in comparison to hysteroscopy were studied. Results: A total of 44 patients completed the study. The average age of the study population was 44.8 years and the mean parity was 1.8. Nulliparas represented 34.03% of the study population and the average duration of symptoms was 14.8 months. Conclusion: In the investigation of women with abnormal bleeding in an outpatient setting, both hysteroscopy and SHSG are comparable in the diagnosis of intra-cavity lesions, pain rating, and procedure time. However patient acceptability of SHSG was significantly more when compared to outpatient hysteroscopy.

Key words: Sonohysterography; Hysteroscopy; Evaluation of uterine cavity; Abnormal uterine bleeding; Outpatient approach.

Introduction

Abnormal uterine bleeding represents 33% [1] of all gynaecological outpatient referrals and this could rise to as much as 69% in the peri- and post-menopausal group [1, 2]. Causes vary from hormonal imbalance, pregnancy-related problems, to focal causes such as fibroids, endometrial and cervical polyps, and endometrial and cervical cancers. Hysteroscopy combined with endometrial biopsy has almost replaced dilatation and curettage for the investigation of this symptom. Though most hysteroscopies are performed under general anaesthetic, there is enough evidence to suggest it is a well-tolerated and acceptable outpatient procedure [3-5].

Transvaginal sonography (TVS), one of the tools in the evaluation of abnormal bleeding, does not always distinguish between certain sonolucent alterations of the endometrium, such as polyps and hyperplasias, or between proliferative phenomena that result from exogenous hormonal therapy. However, when sonographic evaluation follows uterine cavity distension as in sonohysterography (SHSG), the resolution is greatly enhanced, resulting in an impressive increase in diagnostic sensitivity for polyps, myomas, hyperplasias, and foreign bodies [6-10]. SHSG is now more frequently being used in the evaluation of women with gynaecologic conditions since Diechert first reported that TVS detection of uterine lesions could be enhanced by the simultaneous infusion of saline [6]. Three-dimensional (3D) ultrasound is a relatively new investigative technique and offers several advantages over two-dimensional (2D) scanning. Various imaging modes are available and three perpendicular planes displayed simultaneously can be rotated and translated in order to obtain accurate sections and suitable views needed for diagnosis and geometric measurements. SHSG performed with 3D ultrasound could have even more advantages compared to those with conventional 2D ultrasound. It could give more accurate information regarding the location of abnormalities, and using the multiplanar views, polypoid structures can be clearly visualised allowing for the optimal plane to present the pedicle. The surface-rendering mode can also suppress undesirable echoes allowing polypoid structures to be seen in continuity with the endometrial lining. Another advantage is that it allows documentation and storage of volume information of pelvic organs for later review and analysis [11]. This cuts down on the procedure time, enhances patient acceptability, and proves invaluable when planning further treatment for the patient. The aim of this study was to compare transvaginal 3D SHSG and outpatient hysteroscopy with regards to diagnostic accuracy, procedure time, and patient discomfort.

Materials and Methods

This study was undertaken following approval by the local ethics committee. Both procedures were performed by two
Comparison of transvaginal 3D sonohysterography with outpatient hysteroscopy in the evaluation of abnormal uterine bleeding

Out of the 44 cases, two failed (4.54%) having an outpatient hysterectomy and were put on the inpatient list. The technique of performing hysterectomy without the perfunctory insertion of speculum was successful in 30 out of the 44 (68.18%) patients. Fourteen patients required some form of assistance prior to the outpatient hysterectomy either in the form of some local anesthetic, dilatation, or both. Of these, 12 required dilatation with local anesthetic, one required only dilatation, and one local anesthetic without dilatation. It was also interesting to note that seven out of these 14 (50%) were nulliparas and seven (50%) had previous cervical surgery. Therefore neither of these factors i.e. parity or previous surgery, were significant factors in influencing a successful hysterectomy without the use of a speculum.

3D SHSG

As mentioned earlier, one (2.27%) case failed the SHSG and therefore was excluded from the study. It was observed that 3D scanning provided much better views of the cavity in comparison to 2D in 20 out of 44 (45.45%) cases. These were cases that had fibroids in them and in

groups of experienced investigators who were blinded to the results of the other. Patients were recruited from general gynecology clinics and the one-stop menstrual problem clinic. Consent was obtained following recruitment and to obviate any form of selection bias, patients were randomized to have either hysterectomy or SHSG first. Sealed opaque envelopes that were numbered were opened on recruitment and patients were assigned to one of two groups: group 1 hysterectomy followed by sonography; group 2 sonography followed by hysterectomy. Hysteroscopy was considered the gold standard for uterine cavity evaluation and diagnosis of pathology. All women with abnormal uterine bleeding requiring investigation were included. Those with undiagnosed pregnancies, untreated pelvic infections, suspicious or diagnosed pelvic malignancies, uterine size exceeding 14 weeks, cyeisis, and with medical conditions requiring inpatient hysteroscopy, were excluded. 3D SHSG was performed (GM, CK, DE) in the Early Pregnancy Unit using a Voluson E8 ultrasound (General Electric) equipped with a multifrequency vaginal transducer (5 and 7.5 MHz). SHSG was scheduled for the proliferative phase of the menstrual cycle to exclude any false positive findings. After cleansing of the vulva and vagina, a Cusco’s speculum was inserted to visualize the cervix. The cervix was then cleansed and a paediatric Foley’s catheter (8-10F) was threaded through it into the uterine cavity. The balloon of the catheter was inflated with 0.5 - 1 ml of sterile saline to prevent it from being dislodged. The speculum was then removed to allow the transvaginal probe to be inserted. Five to 20 ml of sterile saline was then instilled via the catheter into the uterine cavity, while SHSG was performed in both the 2D and 3D modes. Initially the scan was performed in the longitudinal axis and the transducer moved from side to side to ensure a thorough evaluation of the uterus from one ostium to the other. Unlike diagnostic hysteroscopy, total distension of the cavity was not required and even a thin stripe of fluid allowed adequate evaluation of the cavity. The transducer was then rotated through 90° in the coronal plane and moved from above downwards to enable visualization of the uterus from the fundus down to the endocervical canal. Two-dimensional frontal, sagittal, and coronal views of the endometrial cavity and uterus were obtained separately. Simultaneous studies of the three planes were stored after obtaining sections parallel to the transducer. Following analysis of the stored 2D images of the distended endometrium, 3D ultrasound images were generated. This was possible by the ultrasound’s computer software integration of the three endomtrial planes. The images were then recorded in the computer system for subsequent retrieval and additional processing. Diagnosis was based on the criteria laid down by Parson and Lense [12]. Hysteroscopy was performed (SR, CK, ALM) in the hysteroscopy suite using a 2.7 mm rigid scope with a 30° fore oblique lens (Hamou 1; Karl Storz, Tutlingen, Germany) and an examination sheath of 3 mm. Normal saline was the distension media. The scope was introduced through the vagina into the cervix without inserting a speculum. As the normal saline distended the vagina and the os came into view, the scope was introduced through the cervical canal into the cavity. All procedures were monitored using a video camera and monitor. However, when cervical stenosis and or pain halted the procedure, dilatation of the cervix with or without local anaesthetic infiltration, was carried out. Cervical dilatation to 5 mm diameter was accomplished with the use of graduated Hegar dilators. Local anaesthetic infiltration was carried out using a total of ten mls of Citanest 3% with Octapressin® (3% Prilocaine Hydrochloride 30 mg/ml, Felypressin 0.03 unit/ml, Astra Zeneca) given via a dental syringe. Intracervical injection of this anaesthetic was given at 2, 5, 7, and 11 o’clock positions. A study was judged adequate only when the entire uterine cavity and both tubal ostiae were visualised. An endometrial biopsy using a pipelle endometrial sampler (Laboratoire CCD, Paris) was carried out only when deemed necessary. No premedication was given to the patients before either of the procedures. After each procedure, the patients were asked by an independent examiner to score the pain rating on a visual analogue scale between 0 (none) to 10 (most). Patients were also asked to report any complication or any further discomfort encountered. Time of procedure for the hysteroscopy was defined as starting from initial placement of hysteroscope into the vagina and ending with removal of all equipment; for SHSG, it was time the lapse initiated with speculum placement and ending with simultaneous removal of ultrasound transducer and Foley’s catheter. This was a prospective cohort study which aimed to recruit at least 40 patients (20 in each arm) to give the study a statistical power of 80%.

Results

A total of 49 patients consented to the study of which only 44 completed it: two dropped out after the hysteroscopy due to pain, two had stenosed cervix, and therefore were put on the day surgery hysteroscopy waiting list, and one had a multifibroid uterus, and thus failed the SHSG. The two main reasons for failure in either procedure were stenosed cervix and pain.

The average age of the subject population was 44.8 years with the age range between 26 - 63 years. The young patient was investigated only because she had persistent irregular bleeding per vaginum despite hormonal modulation. The mean parity was 1.8 with nulliparas comprising 34.03% of the study population. The average duration of the symptoms was 14.8 months. The indications for referral to the unit are set out in Table 1 with menorrhagia being the most common.

Hysteroscopy

44.8
one where the uterus was studded with multiple fibroids, 2D was unable to produce a satisfactory picture. In one case, unsatisfactory views were obtained with both 3D and 2D scans as the uterus was enlarged with multiple intramural fibroids.

Comparison of hysteroscopy and 3D SHSG diagnosis

As mentioned, hysteroscopy was considered the gold standard in investigating abnormal uterine bleeding. Intracavity lesions were noted in 26 out of 44 (59%) women presenting with abnormal uterine bleeding. The diagnostic potential of SHSG as compared to hysteroscopy is set out in Table 2.

<table>
<thead>
<tr>
<th>Indications</th>
<th>Polyp</th>
<th>Fibroid</th>
<th>Abnormal</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menorrhagia</td>
<td>23</td>
<td></td>
<td></td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Post-menopausal bleeding</td>
<td>6</td>
<td></td>
<td></td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Metorrhagia</td>
<td>6</td>
<td></td>
<td></td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Fibroids with acyclical bleeds</td>
<td>2</td>
<td></td>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Endocervical polypl</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Subfertility</td>
<td>1</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. — Diagnostic potential of sonohysterography as compared to hysteroscopy.

Duration of procedure

Time taken to perform hysteroscopy varied between one and 13 minutes with a mean of six minutes (standard deviation ± 2.86 with a 95% CI between 5.119 and 6.881). Time taken to perform SHSG varied between 10 and 30 minutes. The mean was 17.29 with a standard deviation of 4.43 and 95% CI between 15.76 and 18.81. On using the Wilcoxon signed rank test to compare the duration between the two procedures, the p value was ≤ 0.001.

Patient satisfaction

This was an important outcome measure in this study. Thirty out of 44 (68.18%) cases felt they would prefer SHSG if given the choice again. In contrast, only seven out of the 44 (15.91%) cases would choose hysteroscopy again. Three of the cases (6.82%) had no specific preference and three cases (6.82%) did not respond to the question. These results show that despite pain being comparable in both the procedures, there was a marked preference for SHSG.

Discussion

Abnormal uterine bleeding generates a diagnostic challenge for the gynaecologist and the ideal diagnostic tool would be an accurate, safe, and easily performed procedure in an office setting. The authors detected a prevalence rate for intracavity lesion of 59% in women with abnormal uterine bleeding. This is similar to the findings of other investigators [6, 10-15]. The present study though small and involving only 44 patients, shows that SHSG is comparable to office hysteroscopy in terms of diagnosis and ease of performance. These findings are similar to those of Gumus II. et al. [9], Widrich T. et al. [13] and other researchers, who compared SHSG and office hysteroscopy in premenopausal women and found nearly identical sensitivity and specificity. Saidi et al. [14] however found SHSG to have higher sensitivity and specificity than hysteroscopy. The authors scheduled the SHSG in the proliferative phase of the cycle in menstruating women to avoid false positive results. However it has been reported that SHSG findings are independent of cyclic endometrial changes [12]. The authors noted that the use of 3D-SHSG allowed a better definition and characterization of focal endometrial thickening and myometrial extensions of submucous fibroids. This property proved useful in mapping fibroids prior to resection and needs to be addressed in a proper randomized trial. A similar study comparing 2D and 3D SHSG in the evaluation of uterine lesions found the latter to be more effective with 100% specificity [16]. The above results show that the SHSG took a much longer time than hysteroscopy with the average time being roughly 17.29 ± 4.43 min. However it is important to point out that this is actually the total duration of procedure time which commences with insertion of the speculum, insertion of the catheter, and transvaginal scanning along with 2D and 3D SHSG.
If 3D SHSG were to be looked at in isolation, the average time from insertion of catheter to completion of procedure was < 5 min which was comparable to that recorded by others [13]. It was surprising to note that pain ratings were comparable for both procedures. This was contrary to that found by other investigators [13] and the present authors believe that the technique used i.e. not inserting a speculum prior to performing the hysteroscopy, is probably responsible for the low pain scores. Unfortunately the comparable pain ratings were not reflected in patient acceptability. Hysteroscopy was considered more invasive and to some extent more of an “operative procedure” than SHSG. This is an issue that needs to be addressed in a larger study. The authors also noted that parity, previous cervical surgery, and post-menopausal status did not significantly influence success at outpatient hysteroscopy.

In summary, this study compares two procedures that are useful in investigating women with abnormal bleeding in an outpatient setting. Both are comparable in the diagnosis of intracavitary lesions, pain rating, and procedure time. This brings to light an important point i.e. in units which lack an outpatient hysteroscopy set up, performing a SHSG in cases where a conventional scan is unable to offer a diagnosis or where an abnormality is suspected, could prove to be just as useful. Besides, to set up a SHSG service, there is no need of a theatre setting with additional theatre staff and therefore could prove to be cost-effective. While the present study, because of its size, could not conclusively prove the advantages of choosing a 3D ultrasound machine over a 2D one, it was clear that in the presence of abnormalities, such as fibroids, the cavity was much easily visualized with the 3D machine. The development of SHSG is a significant advance in gynaecologic investigation and there is a case for incorporating it in gynaecologic scanning services. Therefore in terms of patient acceptability, post-operative complications, visualizing the adnexae, and determining myometrial extension, SHSG scores over hysteroscopy.

References


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Factors affecting completion of laparoscopic myomectomy

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Department of Obstetrics & Gynecology, Kung Hee University Hospital at Gangdong, Kyung Hee University Medical College, Seoul (Korea)

Summary

Purposes: This study aimed to elucidate the factors affecting completion of laparoscopic myomectomy without unintended surgery. Materials and Methods: The medical records of 143 patients who underwent laparoscopic myomectomy desiring to retain their uterus were retrospectively reviewed. Unintended surgery was defined as the need for conversion to other surgical methods including laparotomy or laparoscopic hysterectomy at any time during the procedures. All variables associated with completion of laparoscopic myomectomy in the univariate analysis were selected at the threshold of \( p < 0.25 \) and then tested in a multiple-logistic regression model. Results: The rate of unintended surgery was 13.3%. Univariate analysis revealed that age, previous abdomino-pelvic surgery, current medical disease, transfusion, > five myomas, myoma size > 8.2 cm, posterior wall location of myoma, intramural type of myoma, and the presence of adenomyosis were statistically significant risk factors for unintended surgery. Multivariate logistic regression analysis demonstrated that completion of laparoscopic myomectomy was significantly influenced by a history of previous abdomino-pelvic surgery (odds ratio: 6.46, 95% CI: 0.03-0.41; \( p \) value 0.04). Conclusion: The risk of unintended surgery during laparoscopic myomectomy is associated with a history of previous abdomino-pelvic surgery.

Key words: laparoscopic myomectomy, unintended surgery, completion of laparoscopic myomectomy, adrenal surgery

Introduction

Laparoscopic myomectomy has been regarded as a uterine-conserving procedure that involves only a small scar, less postoperative pain, short hospital stay, fast recovery, and early return to work [1]. However, the complex surgical technique and expertise of the involved surgeon are important limiting factors constraining the widespread use of laparoscopic myomectomy. The indication for surgery, surgical techniques, and associated risks remain debatable issues for laparoscopic myomectomy. The purpose of this retrospective study was to assess the rate of unintended surgery among patients who underwent laparoscopic myomectomy and to elucidate the risk factors affecting completion of laparoscopic myomectomy without unintended surgery.

Materials and Methods

The medical charts of 143 women who underwent laparoscopic myomectomy between January 2007 and August 2010 in a teaching university hospital were reviewed retrospectively. The Institutional review board approval was obtained prior to performing the chart review.

Any symptomatic women who desired a uterus-conserving treatment were included in this study. Inclusion criteria were: the presence of at least one symptomatic myoma with longest dimension of 5-12 cm, fewer than seven myomas as measured by ultrasound examination, and uterine size < 16 gestational weeks by pelvic examination. Preoperative characteristics of the patients are summarized in Table 1. The gonadotropin-releasing hormone agonist (GnRHa) therapy was not given preoperatively in any patient. Prophylactic antibiotics were injected and prostaglandin (estradiol) E2 was administered via the rectum just prior to each operation. A diagnostic hysteroscopy was performed if there was suspicion of endometrial involvement of myoma just prior to laparoscopic myomectomy. Laparoscopic myomectomy was performed by technique described elsewhere [2]. Supramural placement of a primary trocar, or in situ morcellation while the myoma remained attached to the uterus, was applied as necessary when manipulation of the uterus in a limited space was difficult. Diluted pitressin was injected into the myometrium for hemostasis. The enucleated myomas were removed with an electromagnetic morcellation. Sutureing was performed at the sites of deep suberosal or intramural myomas with one to three layers, depending on the depth, with continuous or interrupted 0 polyglactin sutures. Two experienced surgeons performed all the surgical procedures.

Unintended surgery was defined as the need for conversion to other surgical methods including laparotomy or laparoscopic hysterectomy at any time during the procedures, either because of complications or technical difficulties. Patients whose procedure was converted to open surgery or laparoscopic hysterectomy were compared with those of successful laparoscopic myomectomy.

Statistical calculations were performed using SAS statistical software (SAS Institute, Cary NC) and R (version 2.2.0). All the results are expressed as median and range. Variables were compared with Mann-Whitney test and Pearson \( \chi^2 \) test between two groups. The binary variable for the number, size, weight, and location of myoma were decided with the classification and regression tree (CART) analysis. All variables associated with completion of laparoscopic myomectomy in the univariate analysis were selected at a threshold of \( p < 0.25 \) and then tested in a multiple-logistic regression model. In the final logistic regression model, the adjusted odds ratios (OR) and their confidence interval (CI) were calculated from the model’s coefficients and their standard deviation.

Results

Of the 143 patients who were scheduled to receive a laparoscopic myomectomy, 19 (13.3%) patients had...
unintended surgery. The conversion to unintended surgery was related to intraoperative complications and technical difficulties as follows: adenomyosis and pelvic adhesion in nine cases, technical difficulty in five cases, severe pelvic adhesion in four cases, and severe hemorhage in one case. Therefore, seven cases of open myomectomies, six cases of laparoscopically-assisted vaginal hysterectomies, three cases of laparoscopic subtotal hysterectomies, and three cases of total abdominal hysterectomies were performed.

The perioperative characteristics including myomas are summarized in Tables 2 and 3. In the successful laparoscopic myomectomy group, 261 myomas were removed in 123 patients. During dissection of the myoma, the endometrial cavity was opened in 2.3% and adenomyosis was confirmed in 16.3% in the pathology reports.

CART analysis revealed that characteristics of removed myomas that included fewer than five myomas, intramural myomas that included fewer than five myomas, intramural myomas that included fewer than five myomas, intramyometrial myomas, the presence of at least one symptomatic myoma having maximum dimension of nine cm to the lack of influence of size, number, or location of the myoma [1, 8-11]. The indications of laparoscopic myomectomy are

<table>
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<tr>
<th>Table 1. — Preoperative characteristics of the cohort.</th>
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<tbody>
<tr>
<td>Characteristics</td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>44.0 43.0</td>
</tr>
<tr>
<td>Gravidity</td>
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<tr>
<td>Purity</td>
</tr>
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<td>Body mass index (kg/m²)</td>
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Previous abdomino-pelvic surgery: 36/123 (29.3%)
p = 0.04). Postoperative transfusion, > five myomas, myoma size > 8.2 cm, posterior wall location of myoma, intramural type of myoma, and presence of adenomyosis. In the final multivariate logistic regression analysis, the important factor affecting completion of laparoscopic myomectomy was history of abdomino-pelvic surgery (OR: 6.46, 95% CI, 0.03-0.41; p value 0.04).

Discussion

The conversion rate to open surgery during laparoscopic myomectomy varies widely from 0 - 41.4% [3-7]. The reason for variable conversion rate is not clear, and may simply reflect reports of successful laparoscopic myomectomy. In case of failed laparoscopic myomectomy, the alternatives include not only open surgery but also laparoscopic hysterectomy. Therefore, the purpose of this study was to elucidate the rate of unintended surgery (i.e., conversion to other alternatives any time during the procedure), not the rate of open surgery.

The indications of laparoscopic myomectomy are debatable and include limitation in surgical expertise and characteristics of the myoma. However, with increasing surgical expertise, the criteria have grown from less than two to three in number to less than eight, and from a maximum dimension of nine cm to the lack of influence of size, number, or location of the myoma [1, 8-11]. The presence of at least one symptomatic myoma having longest diameter of 5-12 cm, ≤ seven myomas, and ≤ 16 gestational weeks of uterus measured by pelvic examination was pelvic examina-

<table>
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<tr>
<th>Table 2. — Characteristics of the removed myomas.</th>
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<td>Characteristics</td>
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<tr>
<td>Number of removed myomas</td>
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<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>7</td>
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</tbody>
</table>

Type of myoma | 261 | 44 |
| Intramural | 121/261 (46.4%); | 43/44 (79.7%); |
| Subserous | 140/261 (53.6%); | 1/44 (2.3%); |

Entered uterine cavity | 6/261 (2.3%); | 2/44 (2.3%); |

Location of myoma | 261 | 44 |
| Fundus | 26/261 (10.0%); | 7/44 (15.9%); |
| Anterior | 104/261 (39.8%); | 12/44 (27.3%); |
| Posterior | 92/261 (35.2%); | 22/44 (50.0%); |
| Lateral | 13/261 (5.0%); | 2/44 (4.5%); |
| Cervical | 23/261 (8.8%); | 1/44 (2.2%); |

Size of single myoma | 63 | 8 |
| < 60 mm | 21/63 (33.3%); | 3/8 (37.5%); |
| 60-100 mm | 37/63 (58.7%); | 4/8 (50.0%); |
| > 100 mm | 5/63 (7.9%); | 1/8 (12.5%); |

Associated pathology | 123 | 19 |
| Endometriosis | 15/123 (12.2%); | 3/19 (15.8%); |
| Adhesion | 21/123 (17.1%); | 13/19 (68.4%); |
| Adnexal mass | 17/123 (13.8%); | 2/19 (10.5%); |
| Intraligamentary abnormality | 11/123 (8.9%); | 0/19 |

<table>
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<th>Table 3. — Perioperative characteristics of the cohort.</th>
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<tr>
<td>Characteristics</td>
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<tr>
<td>Operation time (minutes)</td>
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<tr>
<td>Change of hematocrit (%) at 1st postoperative day</td>
</tr>
<tr>
<td>Complications</td>
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<tr>
<td>Intraoperative transfusion</td>
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<tr>
<td>Bladder injury</td>
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<tr>
<td>Postoperative transfusion</td>
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<tr>
<td>Pelvic effusion</td>
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<tr>
<td>Paralytic ileus</td>
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<tr>
<td>Febrile morbidity</td>
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* p < 0.05 by Chi-square test; † p < 0.05 by Mann-Whitney test.
tion were included in this study. The characteristics of the myoma have been regarded as important predictors of conversion to open surgery. Dubuisson et al. proposed a prediction model for conversion that comprised myoma size five cm, intramural type, anterior location, and preoperative use of GnRHa [12]. Marret et al. identified the surgeon’s experience as another important risk factor of laparoconversion [13]. Also, they confirmed that size and intramural type of myoma were important risk factors, but not an anterior location. The CART analysis in this study showed significant binary variables between characteristics of myomas and conversion to unintended surgery. The cut-off points included a myoma number of five, size of 82 mm, posterior location, and intramural type. However, these variables disappeared with multivariate logistic regression analysis. The selection criteria based on the characteristics of myoma used in this study seemed not to affect the completion of laparoscopic myomectomy; rather, the surgeon’s expertise may be a more important factor for unintended surgery, which is consistent with other studies [9, 13].

A history of abdomino-pelvic surgery was revealed as the only significant risk factor of unintended surgery. In several studies, previous laparotomy was closely related with increased complications and laparoconversion [14, 15]. The abdominal wall and bowel adhesions are more prevalent and lysis of bowel and abdominal wall requires more surgical skill and expertise of surgeon in such cases of previous laparotomy, contrary to adhesions in the cases of benign gynecologic diseases. Therefore, patients should be informed that they have a higher risk of unintended surgery if they have adhesions.

Although the retrospective study design was a limitation, the suggested inclusion criteria for selecting patients suitable for laparoscopic myomectomy can be used as a useful decision-making tool. Therefore, clinicians and patients can ponder laparoscopic myomectomy on the basis of these findings when minimally-invasive uterine-preserving surgery is the goal.

Conclusion

The risk of unintended surgery during laparoscopic myomectomy is associated with a history of previous abdomino-pelvic surgery.

References


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How to prevent the complications caused by the changes of pelvic anatomical relationship after gynecological surgery?

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Introduction

In recent years, as the standard of living improves, women’s healthcare concerns also grow, causing many gynecological diseases to be diagnosed at an earlier stage and treated in time. As a kind of therapeutic measure, while alleviating pain in female patients, surgery also implicates a series of issues, including diseases and discomforts of various degrees, such as mental and psychological disorders and functional alterations. This paper discusses some diseases caused by changes of pelvic anatomical relationship after gynecological surgeries, as well as their prevention and treatment.

Discussion

Hysterectomy

The uterus is an important female reproductive organ, located at the center of the pelvic cavity and between the urinary bladder and rectum, connected to the vagina at its lower part, having a uterine tube, and an ovary on both sides. Depending on the supports of the uterine ligament, pelvic floor muscles, and fascia, the uterus maintains its antversion and anteflexion and performs important physiological functions [1]. For benign uterine diseases requiring surgical treatment, according to the patient’s specific conditions, the adoptable surgical methods mainly consist in subtotal hysterectomy and hysterectomy; the former reserves the cervix of the uterus, uterosacral, and cardinal ligaments [2]. While a hysterectomy cures the previous disease, other following diseases may result due to the loss of the uterus and resulting change of anatomical positions of other organs around the pelvic floor.

1) Prolapse of the ovary

While carrying out hysterectomy or subtotal hysterectomy reserving the ovary, the left ovary is not fixed, and some patients with a long infundibulopelvic ligament may show prolapse or torsion, resulting in pain or discomfort. The pain is periodic or continuous, and is mostly dull, or non-radiating to the lower abdominal or lumbo-costal regions.

Generally, pain caused by prolapse of the ovary is mild but cannot be treated. Prevention includes a good knowledge of pelvic anatomy, while fixating the ovaries on both sides and embedding the residual ends to avoid ovarian prolapse or torsion.

2) Prolapse of vaginal or cervical residual end

The female pelvic floor is comprised of multiple layers of muscles and fasciae closing the pelvic outlet, and the urethra, vagina, and rectum penetrate through it. The pelvic floor muscle group, fasciae, ligaments, and their nerves form a complex pelvic floor support system and sustain the pelvic organs at their proper positions, such as the uterus, urinary bladder, and rectum.

Under normal conditions, the uterosacral and cardinal ligaments together perform the function of maintaining the position of the uterus, and the round ligament possesses the function of preventing its anteverision. After hysterectomy, the vaginal residual end loses the pulling and drawing action of the uterosacral, cardinal, and round ligaments; with subtotal hysterectomy, if the round ligament is not fixed to the cervical residual end, the reserved cervix of the uterus loses the pulling and drawing action of the round ligament; especially, when there are inducing factors of prolapse (such as long-term standing, increase of abdominal pressure, hard physical labor, and long-term cough), prolapse of vaginal or cervical residual ends may occur [3-5].

Prevention and treatment methods include: (a) mastering of the surgical indications, selection of proper surgical methods, and avoiding excessive treatment; (b) for

Summary

Gynecological surgery may bring about a series of corresponding diseases, because the excision of certain organs will have changed the pelvic anatomical relationship. The gynecologist must be well aware of the surgical indications for various diseases and select the proper method, range, and approach to achieve the optimal therapeutic effects with minimal injuries. This paper discusses some diseases caused by changes of pelvic anatomical relationship after gynecological surgeries, as well as their prevention and treatment.

Key words: Gynecological surgery; Pelvic anatomy; Complications; Prevention.
hysterectomy patients, the round ligament can be suspended on the vaginal or cervical residual end; however, an incorrect suspension of the round ligament, such as an improper grasp and too tight a suspension may cause the patient lower abdominal discomfort or pain. With surgical indications permitting, an intraperitoneal hysterectomy should be carried out as much as possible; (c) reserve the ovary or ovarian tissues as much as possible, to maintain the normal body hormone level, and reduce the prolapse of genital meatus caused by lack of hormone; (d) for patients showing the prolapse trend, while carrying out the suspension of round ligament, shortening of uterosacral ligament should also be included; (e) if the cause is lack of estrogen, under the condition of free of contraindications, the estrogen preparation should be applied locally; (f) for patients with serious prolapse, surgical treatment is indicated.

3) Prolapse of the Fallopian tube

Prolapse of the Fallopian tube, a rare complicating disease of hysterectomy, means that the Fallopian tube falls off into the vaginal residual end. It occurs most frequently with vaginal hysterectomy than during transabdominal surgery. If it has occurred within several months after hysterectomy, it is referred to as early-stage prolapse, or later-stage prolapse if several years have elapsed. The common clinical symptoms of prolapse of the Fallopian tube include: watery or blood-watery vaginal discharge, painful sexual intercourse, contact bleeding, and lower abdominal pain [6]. During examination, a polyp-like red tissue can be seen at the vaginal residual end; while palpating or pulling, the lower abdominal pain occurs, and the mass can be palpated at the top of the vagina. In 2006, the Peking Union Medical College Hospital reported that among 7,949 patients with a hysterectomy performed in that hospital during the last 20 years, the incidence rate of Fallopian tube prolapse was 0.11% - lower than 1.3% overseas; it was 0.08% for laparotomy, and 0.51% for vaginal hysterectomy; there was no single case of vaginal hysterectomy performed laparoscopically [7].

The most common reason for Fallopian tube prolapse is that during hysterectomy, the residual ends of adnexa and round ligament are sutured to the vaginal residual end, translating the Fallopian tube closer to the vaginal residual end; the vaginal residual end is open or the drainage tube is placed, causing infection of vaginal residual end, non-healing, dehiscence, Fallopian tube adhesion, fallout of fimbria; while pulling out the drainage tube, there is a risk that the fimbria tip is also drawn out. The prolapse of the Fallopian tube should be distinguished from vaginal adenosis, granulation tissue of vaginal residual end, and from tubal endometriosis.

Prevention methods include: (a) after laparotomy and carrying out hysterectomy, fixating the adnexa on the side wall of the pelvis; (b) carrying out peritonization of vaginal residual end during surgery can avoid Fallopian tube prolapse; (c) after surgery, if the vaginal drainage tube is applied, it should be placed in the retroperitoneal gap and in the residual vaginal end; (d) while removing the vaginal drainage tube, the patient should raise her buttocks, and rest for 1-2 hours after its removal, and then made to walk to avoid the Fallopian tube from prolapsing into the extraperitoneal gap.

Treatment methods include: for the vaginal fallage, the exposed tube should not only be resected, but the vaginal residual end should also be reopened while freeing it completely, and then retracted and resecting the entire Fallopian tube.

4) Change of vaginal structure

For patients with hysterectomy, there is no cervix of the uterus on top of the vagina and the surgical technique sutures the anterior and posterior vaginal fornix; since the cervix of uterus is resected and the anatomical integrity of vagina is damaged, the length of the vagina is shortened to some degree. As known that during sexual intercourse, the cervix of the uterus plays the role as orgasm trigger, the pressing of cervix of the uterus causes the pendular movement of uterus and ligament, and stimulates the uterus to contract and surrounding peritoneum to produce pleasure; when the uterus and cervix of uterus are resected, the anatomical structure of the vagina is altered, causing the reduction of such stimulus, and consequently the reduction of quality of sexual life after surgery [8, 9].

After hysterectomy, the vaginal top innervation of the uterus and integrity of the pelvic floor are damaged, causing the innervation at the upper vaginal section, intestinal tract, urinary bladder, and these areas to be damaged, while reducing pleasure during sexual intercourse; at the same time, since the sources of leucorrhea decrease (body of uterus, cervix of uterus), the vagina becomes dry, resulting in the discomfort of sexual activity.

For subtotal hysterectomy maintaining the cervix of the uterus, although the vaginal structure does not change in theory, because of the reduction of sources of secretion and loss of secretion from the endometrium, as well as the loss of supporting function to women’s orgasm from the contraction of uterine smooth muscles, the quality of sexual life will be affected to varying degrees [10]. Ayoubi [11] discovered from questionnaires that the quality of sexual life of 60.4% patients with hysterectomy did not change, and in 18.3% it was reduced; among them, the influence upon patients’ sexual function from laparoscopic hysterectomy and vaginal hysterectomy is less than that from laparatomized hysterectomy. Additionally, some reports stated that there was no statistical difference in the quality of sexual life after hysterectomy and subtotal hysterectomy, which may derive from the surgical skills and patients’ psychological factors [12].

Of course, the quality of sexual life is also related to the patient psychological factors. Therefore, before carrying out therapeutic measures, good communication with the patients should address the issues that may result
from anatomical and psychology postoperative changes to reduce patient distress; heteropathy, as well as professional psychological treatment should be sought, if necessary. For patients experiencing a margin of other therapeutic methods, humanized services, i.e., under the condition of reaching curative purpose, respecting the patient’s will to select an alternative method, and including whether to undergo or not surgery, should be provided.

**Adnexectomy**

The ovary is a major organ that maintains female endocrine activities and reserves a series of important functions in the human body. Estrogen is mainly produced by the ovaries; if ovarian activity is incomplete or lost, besides the influence upon normal sexual function, various disease symptoms due to low level of estrogen may follow, such as vasomotor dysfunction, osteoporosis, lipid metabolism disorder, cardiovascular diseases, sexual organ atrophy, etc., affecting the patients’ quality of life.

1) **Bilateral adnexectomy**

Existing research shows that among women’s genitourinary tract tissues, there are estrogen receptors (ERs) in the cardinal and uterosacral ligaments, levator ani muscle, posterior vaginal fornix, and in the vaginal wall tissue; the strength of ER expression is influenced by the level of estrogen. Postsurgical research on the macaques with ovariectomy showed that the reduction of tension of elastic fibers of tissues on both sides of the vagina, including collagen, and levator ani muscle, causes the chalasis of the pelvic floor tissues [13]. When bilateral adnexectomy is performed, hysterectomy is often carried out simultaneously; therefore, the prolapse of pelvic floor organs is more likely to occur. Due to the lack of estrogen, a series of symptoms, such as hyperhydrosis, hectic fever, emotional fluctuation, vaginal dryness, pain, etc., may take place, affecting the quality of postsurgical life [14].

2) **Benign ovarian diseases (cyst or tumor or other focuses), focus excision or unilateral adnexectomy**

The factors which may affect ovarian blood circulation and function include: excessive removal of normal ovarian tissue, electrocoagulation after laparoscopic focus excision without suture, damage to cortex of the ovary, lesser amount of residual normal ovarian tissue due to heavy focus, too dense suturing of residual ovarian tissue, etc.

The following methods can be used to prevent or reduce such issues: (a) for the patient requiring bilateral ovariectomy: the surgical indications should be strictly managed to avoid excessive treatment; for the patient with single ovariectomy: hormonal replacement therapy should be prescribed; (b) for hysterectomy patients with ovary preservation: the uterine tube and natural ligament should be spared as much as possible; for the young patient with uterine myoma, even if child-bearing has been completed, myomectomy should be performed according to the patient’s will and preserve the uterus; (c) for the pre-menopausal patient with benign ovarian disease: focal excision should be performed while preserving normal ovarian tissue as much as possible, and special attention should be paid not to damage the hilus vessel of the ovary. While performing laparoscopic surgery, the suture method should be adopted as much as possible; when electrocoagulation is a necessary, it should be performed accurately to avoid treating an unreasonable large-area; the residual ovarian tissue should not be sutured too densely as long as hemorrhage has been arrested.

In summary, since the changes of pelvic anatomical relationships after gynecological surgery will have a negative influence of varying degrees in multiple aspects of a women’s life, as well as bringing about several possible complications, clinicians should master the surgical indications, select the proper method, range, and approach, according to the patient’s age and requests, as well as carefully access the disease type and severity. Surgery should be as minimally invasive as possible to achieve optimal therapeutic effects, while reducing the long-term associated diseases as much as possible, which will be beneficial to the patient’s physical and mental health, as well as improving the quality of life.

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Bilateral hypogastric artery ligation in emergency setting for intractable postpartum hemorrhage: a secondary care center experience

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¹Anatolia IVF Center, Ankara; ²Middle East Health Center, Sanliurfa (Turkey)

Summary

Objective: To report the authors' experience in bilateral hypogastric (internal iliac) artery ligation which was performed for controlling intractable postpartum hemorrhage in a secondary care center. Materials and Methods: The patients that required bilateral hypogastric artery ligation for severe intractable postpartum hemorrhage from November 2007 to August 2009 were included in this retrospective study. Data were retrieved from patients’ hospital records. Results: A total of 26 cases required hypogastric artery ligation during the study period. Causes of postpartum hemorrhage included uterine atony, placental abruption, uterine rupture, and placenta accreta. Hemorrhage was effectively controlled in 20 of 26 cases (76.9%) and hysterectomy was avoided. Iliac vein injury occurred in one patient (3.8%) as an operative complication. There was one maternal death. Conclusion: Hypogastric artery ligation is an effective therapeutic option for severe postpartum hemorrhage and should be kept in mind during obstetric emergency conditions.

Key words: Hypogastric artery ligation; Postpartum hemorrhage; Peripartum hysterectomy.

Introduction

Primary postpartum hemorrhage (PPH) is a common cause of maternal morbidity with sequel such as renal failure, acute respiratory distress, coagulopathy, and Sheehan syndrome. PPH is also one of the top five causes of maternal mortality in both developing and developed countries, where maternal mortality is both about one hundred times higher in developing countries [1].

Risk factors for PPH include: placental abruption, placenta previa, antepartum hemorrhage, previous PPH, preeclampsia, multiple pregnancies, induction of labor, augmentation of labor, instrumental deliveries, perineal tear, high birth weight, and retained placenta [2]. However, only a small proportion of women with any risk factors develop PPH and many women without risk factors experience hemorrhage after delivery; thus, knowledge of risk factors is not clinically very useful.

PPH treatment includes resuscitation and treatment of etiologic causes such as fundal massage, uterotonic agents, suturing tears, and removal of the placenta. If the patient does not respond to initial management, surgical interventions such as uterine tamponade procedures, uterine compression sutures, uterine artery ligation, bilateral hypogastric artery ligation (BHAL), X-ray guided artery embolisation and/or hysterectomy should be considered [3]. The choice will depend on future fertility desire of the patient and experience of the surgeon.

Bilateral hypogastric artery ligation was first described by Kelly in 1894 and has been advocated in the management of intractable PPH and in the prevention of maternal death [4]. Hypogastric artery ligation does not lead to complete cessation of blood flow to pelvic organs. Bleeding from the uterus diminishes because arterial pressure or pulsation drops but pelvic circulation transforms into a venous system [5]. Venous bleeding can usually be controlled by temporary pressure, so that a blood clot could form at this site and has a good chance of remaining there [6].

In the case of intractable PPH, hypogastric artery ligation does not only save the life of the patient but also preserves fertility. Recent reports have shown that this procedure does not impair subsequent fertility and pregnancy outcomes [7, 8]. However, the success rate of this procedure ranges between 40-100% and the procedure prevents 50% of the patients from undergoing hysterectomy [9, 10]. Today, hypogastric artery ligation is performed less frequently than in the past years because practitioners are less familiar with this technique and clinicians fear using it in emergency settings due to possible complications. Additionally, some cultural factors may oblige clinicians not to perform the most effective therapy (such as hysterectomy) for PPH. In order to avoid related medico-legal problems, all clinican should have the ability to perform BHAL.

This study reports the indications and outcomes of 26 cases of bilateral hypogastric artery ligation performed at a secondary care hospital.

Materials and Methods

The study was carried out from November 2007 to August 2009 in Sanliurfa Maternity Hospital, which is a busy country...
maternity hospital. In the study period, there were 34,458 deliveries including 24,492 vaginal (71.1%) and 9,966 Cesarean (29.9%) deliveries. There were 26 BHAL for intractable primary PPH.

The cases were identified by searching operating room records. Patient files were then retrieved from the hospital records. Information regarding specific clinical variables such as age, gravidity, parity, obstetric history, any maternal complications during pregnancy, intrapartum care, delivery type, amount of blood loss, management of postpartum hemorrhage, indication for BHAL, and intraoperative and postoperative complications were obtained from operating room records and patient files.

Criteria for inclusion of patients in the study were: requirement of BHAL procedure for intractable PPH and desire of fertility preservation. Patients who required BHAL after peripartum hysterectomy for PPH were excluded from the study.

Patients who required BHAL for uterine atony were initially treated with uterine massage and uterotonic agents (intravenous infusion of oxitocin up to 60 IU, intramuscular ergometrine 0.2 mg up to five doses and rectal 800 μg misoprostol).

The same technique, other than conventional technique, was used in all cases, as described below. A pfannenstiel incision was used for laparotomy. An incision was made to the posterior peritoneum and the anatomic structures were identified with special reference to the ureter (Figure 1). Fat and loose connective tissue around the hypogastric artery and vein were removed and a right-angle clamp was passed beneath the artery. Using a non-absorbable suture applied to the two cm distal end of the hypogastric artery origin and double-ligated (Figure 2). All of the cases were performed by three surgeons (K.B., I.B., H.G.).

Results

In all 26 cases, the need for BHAL procedure was massive PPH. Mean age, gravida, and parity of the women were 25.6 ± 5.2, 2.9 ± 1.9, and 1.8 ± 1.8 respectively.

Fifteen of 26 cases of BHAL were performed after Cesarean section (C/S) and 11 of 26 BHAL were performed after vaginal delivery. Uterine atony was the most common indication for therapeutic BHAL (69.1%). In 11 of 18 cases, uterine atony was determined after vaginal delivery and seven had C/S. Other indications were placental abruption (6), placenta accreta (1), and uterine rupture (1) (Table 1).

In women with PPH, BHAL was performed primarily with C/S (14), at an interval after C/S (1) or at an interval after delivery [11]. Hemorrhage was effectively controlled in 20 of 26 cases (23.1%) and six women required a hysterectomy (76.9%) (Table 2).

An operative complication occurred in one of 26 women. Injury to the iliac vein occurred in one woman and was repaired by passing a figure-eight prolene 4-0 gauge suture around the defect.

Postoperatively six of 26 women were referred to a tertiary center. Disseminated intravascular coagulopathy occurred in five of these six women. Out of referred six patients, mean transferred packed red blood cells and fresh frozen plasma were 4.3 ± 1.9 and 3.2 ± 1.5 units, respectively.

There was one maternal death in this series. C/S was performed in the patient as indicated for labor dystocia. After the operation, severe PPH due to uterine atony occurred. Uterine massage was performed and uterotonic agents (intravenous infusion of oxytocin up to 60 IU, intramuscular ergometrine 0.2 mg up to five doses, and rectal 800 gr misoprostol) were given to the patient but hemorrhage could not be controlled. Relaparatomy for BHAL was performed after a 45-minute interval, but bleeding did not cease, and thus a hysterectomy was carried out. After the operation, disseminated intravascular coagulation (DIC) was revealed. The patient was referred to a tertiary center and she received a total of 12 units of red blood cells and 11 units of fresh frozen plasma but died 17 hours after the operation.

Twenty of 26 patients that were followed postoperatively in the present hospital, were admitted to the postanesthesia care unit. The mean follow up and mean hospitalization period for these patients were 21.8 hours and 4.4 days, respectively.
Table 1. — Indication for BHAL.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Number of women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine atony</td>
<td>18 (69.2)</td>
</tr>
<tr>
<td>After cesarean section</td>
<td>7 (26.9)</td>
</tr>
<tr>
<td>After vaginal delivery</td>
<td>11 (42.3)</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>6 (23.1)</td>
</tr>
<tr>
<td>Placenta accreta</td>
<td>1 (3.85)</td>
</tr>
<tr>
<td>Uterine rupture</td>
<td>1 (3.85)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100)</td>
</tr>
</tbody>
</table>

Table 2. — Hysterectomy in women undergoing BHAL for PPH.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Total number of women</th>
<th>Hysterectomy carried out n (%)</th>
<th>Uterine salvage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine atony</td>
<td>18</td>
<td>4 (22.2)</td>
<td>77.8</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>6</td>
<td>2 (33.3)</td>
<td>66.7</td>
</tr>
<tr>
<td>Placenta accreta</td>
<td>1</td>
<td>0 (0)</td>
<td>100</td>
</tr>
<tr>
<td>Uterine rupture</td>
<td>1</td>
<td>0 (0)</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>6 (23.1)</td>
<td>76.9</td>
</tr>
</tbody>
</table>

In 26 cases there was no postoperative buttock claudication or necrosis. Two of the 26 patients had wound infection and one of the 26 patients had postoperative ileus. All of these patients did not have systematic follow-up for fertility results after BHAL.

Discussion

BHAL is not only a life-saving procedure but also has the advantage of preserving fertility with no complication in subsequent pregnancy as described in large case series [7]. A major proportion of the blood supply to the pelvic viscera is by the branches of the hypogastric artery. BHAL diminishes the pulsatile pressure of the arterial system and converts it to a venous-like system. This facilitates clot formation and reduces bleeding [10].

The authors believe that BHAL was under-utilized in the management of PPH due to the fear of injury to the iliac veins. The internal iliac vein lies directly posterior to the hypogastric artery. If a controlled manner is performed while passing the right-angled clamp, perforating the underlying internal iliac vein should be prevented. Dissecting the surrounding fascia of the hypogastric artery for passage of the right-angled clamp diminishes the risk of injury to the internal iliac vein. Joshi et al. believe that passing the clamp from lateral to medial side is safer [11]. In this case series, only one internal iliac vein injury occurred. In this case, internal iliac vein was postero-medial to the hypogastric artery and the tip of the clamp injured the vein. Anatomic variations should be considered and more attention should be paid.

The reported success rate of BHAL varies from 40% to 100%, and the procedure averts hysterectomy in only 50% of cases [9, 10]. Failures were more commonly reported in the atomic PPH than in other causes of PPH [12]. In this case series, the uterine salvage rate was 76.9%. Among 18 women with uterine atony who underwent BHAL, four required hysterectomy, resulting in a salvage rate of 77.8%. An early resort to BHAL is thought to be the key to prevent hysterectomy in women with uterine atony [11].

Alternative procedures such as uterine compression sutures, uterine artery ligation, and X-ray guided artery embolisation have some limitations. Selective artery embolisation is an option in managing PPH if the patient is hemodynamically stable but skilled interventional radiologist and the radiologic set-up in proximity is requested [13]. Uterine artery ligation is a promising technique in the management of PPH as occlusion of the uterine artery reduces 90% of the blood flow. It is useful in uterine atony, but in uterine trauma when the avulsed uterine artery retracts into the broad ligament forming a hematoma, it is difficult to perform a uterine artery ligation and salvage the uterus. In cases of deep fornical tears and placenta previa, a significant proportion of bleeding occurs from descending cervical and vaginal artery [11, 14]. In these circumstances, BHAL is more effective by diminishing the blood flow in the uterine, cervical, and vaginal vessels [11]. The B-Lynch suture has been reported to successfully control refractory uterine bleeding in several case series [15]. It can only be used to achieve haemostasis in atomic PPH and is less useful in placenta previa. It finds no application in uterine rupture or bleeding from vaginal lacerations.

The neonatal mortality rate and total fertility rate (births per woman between the ages of 15-49) in South-east Anatolian region were around 21 per 1,000 and 4.19, respectively. Fifty-seven percent of women received ante-natal care from a physician in this region [16]. According to these statistics, this population is at risk for obstetrical complications. Also fertility-saving is very important for this population, therefore BHAL is a good alternative for these patients. This study determined that intraoperative complication rate is low (only one in 26 patients) and it can be feasible for a secondary care center.

In conclusion, BHAL is an effective procedure for PPH and has the advantage of preserving fertility. It can be used in all causes of PPH. Complications related to the procedure are rare and a controlled manner can minimize them. An understanding of retroperitoneal structures and topography should be an integral part of obstetric and gynecological training.

References


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Loss of heterozygosity in the fragile histidine triad (FHIT) locus and expression analysis of FHIT protein in patients with breast disorders

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Introduction

Fragile histidine triad (FHIT) is a tumor suppressor gene that is probably involved with cellular and apoptotic growth and proliferation [1], but the molecular mechanism through which FHIT functions is still not clear [2]. The FHIT gene is composed of ten exons, of which five (exons 5 to 9) code a small mRNA (1.1kb) which is translated to the FHIT protein with 16.8 kDa. FHIT acts in vitro as a typical diadenosine-triphosphate-hydrolase enzyme (Ap3A); however, its enzymatic function in vivo has not been demonstrated [3, 4].

Loss of heterozygosity (LOH) of the FHIT gene has been observed with different frequency in sporadic breast cancer and pre-neoplastic lesions [5-7], suggesting that changes in this gene may be a precocious event in the development of breast cancer. The lobular and ductal epithelia intensely and constantly express the FHIT protein, although, in the majority of carcinomas, a complete loss or a significant reduction in the expression of this protein can be observed [8, 9].

Summary

Purpose of investigation: The fragile histidine triad (FHIT) gene is a tumor suppressor frequently inactivated in various types of tumors. The authors evaluated the occurrence of loss of heterozygosity (LOH) in the FHIT locus and FHIT protein changes in breast tissue. Materials and Methods: Blood and breast tissue samples were obtained from 35 women with mammary disorders. The occurrence of LOH in FHIT locus was assayed by polymerase chain reaction (PCR), and the results obtained from blood and breast tissues from each patient were compared. FHIT protein expression was evaluated by immunohistochemistry. Results: LOH in the FHIT gene occurred in 48.6% (17/35) of patients with mammary disorder. Among patients with malignant breast disorders, 59.1% (13/22) presented LOH in the FHIT gene in comparison with patients with benign breast lumps, in which the LOH was observed in 30.8% (4/13) of women, suggesting that changes in this gene occur prior to the process of mammary carcinogenesis. The changes in the locus of the FHIT gene occur with greater frequency in the coded region of the gene, principally near exons 5 and 8, where the FRA3B site and the histidine triad respectively are found. Changes in FHIT did not modify protein expression. The association between menopause and LOH in the FHIT gene was evident. Conclusions: LOH in the FHIT gene may be related to menopause in women with breast disorders.

Key words: Breast neoplasm; Loss of heterozygosity; Fragile histidine triad protein [supplementary concept]; Tumor Suppressor Gene; Menopause.
Materials and Methods

Patients

A cross-sectional study was carried out involving women with mammary disorders treated at the Mastology Clinic (IPON) of a public hospital school in Uberaba, MG (Brazil), between June 2005 and May 2007. This study was approved by the Committee on Research Ethics of the Universidade Federal do Triângulo Mineiro (UFTM) (CEP 591/2005).

Thirty-five women with breast disorders, in which 22 (62.9%) patients with a diagnosis of breast cancer and 13 (37.1%) women with benign breast lumps were selected. The demographic (age, ethnicity, and family history of breast cancer), clinical (menarche, menopause, and first gestation), and pathological data (type of breast disorder and histological characterization) of these patients submitted to mammary surgery is summarized in Tables 1 and 2.

The majority of the patients had FIGO (Federation International of Gynecology and Obstetrics) Stage II, without the axillary lymph nodes being compromised. A great number of the carcinomas were positive for the estrogen receptor and for the progesterone receptor. Nevertheless, the tumors had a low proliferation index, since MIB-1 expression was low.

DNA purification

The samples of the mammary lesion obtained were fresh fragments taken for histopathological diagnosis and originated from biopsies or surgery. These fragments were washed in a physiological solution and frozen in a -80ºC freezer until the moment of DNA extraction, using the sodium hydroxide smoothing technique [10]. Five milliliters of peripheral blood were also collected with ethylenediaminetetraacetic acid (EDTA), from which the leukocyte DNA was extracted using the phenol-chloroform technique [11].

Polymerase chain reaction

For the polymerase chain reaction (PCR), the authors used microsatellite markers within the FHIT gene: D3S4260 (intron 3), D3S2757 (intron 4), D3S1300 (intron 5), D3S1234 (intron 8) (Table 3). An amplification of the b-globin gene was performed as quality control for the DNA samples. Each microsatellite was amplified using samples of genomic DNA from the breast tissue, as well as blood tissue. PCR protocols were performed as described previously [12] with adaptations. Reactions were prepared in a final volume of 20 μl containing 10 ng of genomic DNA, PCR buffer 1X (10 mM Tris-HCl pH 9.0; and 75 mM KCl), 1.5 mM MgCl2, 200 mM of dNTPs, 750 nM of each primer, and 1.0 unit of Taq DNA polymerase (Invitrogen, São Paulo, Brazil). PCR conditions consisted of an initial denaturation of 5 minutes at 94°C, followed 35 amplification cycles (94°C for 30 s; 48-58°C for 30 s; 72°C for 30 s) ending with a final extension at 72°C for 10 minutes (Table 2). Aliquots of 5 μl of PCR product were loaded in 7.5% polyacrylamide gel and subjected to electrophoresis at 100V over 3-4 hours. DNA bands were observed by silver staining [13].

LOH analysis involved comparing the intensity of the amplified bands corresponding to the alleles of the FHIT gene of the DNA of normal tissue (blood tissue) and tumor tissue (breast tissue) of each patient.

Immunohistochemistry

Samples of breast tissue in blocks of paraffin were used to prepare the slides for immunohistochemistry, using the streptavidin-biotin-peroxidase technique to evaluate the protein expression of FHIT using anti-GST-FHIT rabbit polyclonal antibody material and secondary biotinylate. The slides were

Table 1. — Characterization of patients with breast disorders submitted to mammary surgery according to the demographic, clinical, and pathological variables.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>48.7 years</td>
<td></td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>18</td>
<td>51.4</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>22</td>
<td>62.9</td>
</tr>
<tr>
<td>Black</td>
<td>13</td>
<td>37.1</td>
</tr>
<tr>
<td>Menarche</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early (≤ 12 years)</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Normal (13-15 years)</td>
<td>12</td>
<td>34.3</td>
</tr>
<tr>
<td>Late (≥ 16 years)</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Non-informed</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Menopause</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>51.4</td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>45.7</td>
</tr>
<tr>
<td>Non-informed</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>First gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>4</td>
<td>11.4</td>
</tr>
<tr>
<td>&lt; 30 years</td>
<td>24</td>
<td>68.6</td>
</tr>
<tr>
<td>≥ 30 years</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>Non-informed</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>65.7</td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>28.6</td>
</tr>
<tr>
<td>Non-informed</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>77.1</td>
</tr>
<tr>
<td>Non-evaluated</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>22.9</td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
<td>68.6</td>
</tr>
<tr>
<td>Non-evaluated</td>
<td>3</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Table 2. — Distribution of women with breast disorders according to histological type and Stage.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign - 13/35 (37.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>7</td>
<td>20.0</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>4</td>
<td>11.4</td>
</tr>
<tr>
<td>without atypias</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoma</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Myoid metaplasia</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Malignant - 22/35 (62.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Stage I invasive ductal carcinoma</td>
<td>8</td>
<td>22.9</td>
</tr>
<tr>
<td>Stage II invasive ductal carcinoma</td>
<td>11</td>
<td>31.4</td>
</tr>
<tr>
<td>Stage III invasive ductal carcinoma</td>
<td>2</td>
<td>5.7</td>
</tr>
</tbody>
</table>

*FIGO Stage (Federation International of Gynecology and Obstetrics).
classified according to the intensity of marking as negative, weak positive (+ or ++) and strong positive (+++ or ++++). Immunohistochemical reactions were also performed for the previously-described breast cancer tumor markers MIB-1, estrogen receptor, and progesterone receptor.

**Statistical analysis**

The demographic and clinical data and the results of immunohistochemistry assays were submitted for association analysis with the LOH at FHIT gene. The statistical tests applied were the chi-square test or, when appropriate, the chi-square with Yates correction or Fisher’s exact test. Furthermore, the strength of association was quantified using the odds ratio with 95% confidence interval.

Aiming at the identification of variables that could independently determine the loss of heterozygosity at FHIT gene, a multivariate logistic regression analysis was applied to calculate the adjusted odds ratio, by the inclusion of all studied variables.

Statistical tests were performed using Statistica software version 8.0 and SPSS 17.0 and *p* values of < 0.05 were considered significant.

**Results**

**Analysis of LOH on the locus of the FHIT gene**

The results of the molecular analysis of the FHIT gene are summarized in Table 3. In the non-translated region of the FHIT gene, where D3S2757 and D3S4260 microsatellite markers are located, LOH was observed in 8.6% (3/35) patients in the first and in the last, the retention of heterozygosity was 100% (LOH = 0/35). In the coding region, a higher rate of LOH was observed than in the non-coded region. In the D3S1300 marker region, changes were detected in 8/35 (22.9%) patients. A higher rate of polymorphism between the different DNA samples was found in the region of the D3S1234 marker, where changes were observed in 31.3% (10/32) patients. Analyzing LOH in the whole studied extension of the FHIT gene, combining the results of all markers, the authors observed the loss of one allele in at least one marker in 48.6% (17/35) patients.

**Evaluation of FHIT protein expression by immunohistochemistry**

Analysis of FHIT protein expression is shown in Table 4. Among the patients with loss of heterozygosity, 94.1% (16/17) women strongly-expressed FHIT protein, and only one patient presented an absence of protein expression. Likewise, among the patients without LOH in the FHIT gene, 93.3% (14/15) patients strongly-expressed the FHIT protein and in 1/15 (6.7%) patient a null expression of FHIT protein was found without visible changes in the gene.

**LOH in FHIT gene and its association with risk factors**

Among the patients with benign mammary disorders, 30.8% (4/13) women presented FHIT gene alteration. In comparison, the authors observed a higher rate (59.1% = 13/22) of LOH in patients with malignant disorders, despite the non-significant level (*p* = 0.105).

Likewise, the authors performed a univariate association analysis between LOH at FHIT gene locus and the presence of risk factors for developing breast cancer (Table 5). A significant association (*p* = 0.006) was reported between menopause and LOH at FHIT gene. The majority (12/16 = 75.0%) of women in post-menopausal stage presented LOH at FHIT gene, while just 27.8% (5/18) of women in premenopausal stage exhibited alterations in FHIT gene. This finding, not described in previous studies, represents a chance 7.80 (95% confidence interval, CI = 1.69 - 36.06) times greater for women at post-menopausal stage to exhibit LOH at FHIT gene compared to premenopausal women.

The other demographic and clinical variables did not present a significant association with the occurrence of alteration in FHIT gene. Nonetheless, the authors performed a multivariate analysis aimed in the identification of risk factors that could be independently associated with loss of heterozygosity and to confirm the effect of menopause on alteration in FHIT gene. Regarding this multivariate analysis, any variable was included in the final model of logistic regression, in spite of menopause, which still remained as a determinant factor for the occurr-
rence of changes in FHIT gene with an adjusted odds ratio of 5.40 (95% CI = 1.12 - 26.05) (p = 0.036).

**Discussion**

Genetic deletions in the region of the FHIT tumor suppressor gene is frequently related to the development of malignant cells, including breast carcinomas, whose rate of deletion in the FHIT gene is approximately 30% of the cases. In addition, the reduced or lack of expression of the FHIT protein has been associated with a poorer patient prognosis [8, 14].

The authors evaluated the integrity in the locus of FHIT gene in 35 women with breast diseases, in which the loss of heterozygosity was observed in 48.6% of patients. Similar frequencies were observed when individuals with sporadic breast cancer were evaluated about FHIT gene, in which the LOH was observed in 49.0% of the studied patients [15]. Lower frequencies were found in a study conducted in the south of Brazil, where the authors found intragenic changes in FHIT in six out of 25 cases (24%), using only the D3S1300 marker [16]. The difference between these frequencies may be explained by the fact that the current authors used four different markers in the present work.

A higher rate of LOH was found in the markers located between the coded exons of the FHIT gene, specifically in the regions near to exons 5 and 8. The most fragile site of the human genome, the FRA3B, is located near the region of exon 5. Many authors have discussed the relationship of the FHIT gene to the process of carcinogenesis, raising the idea that a simple change in the fragile site located in the same region is sufficient for cellular transformation and, thus, placing doubt on the tumor suppressor role of the FHIT gene. However, the complementation of the FHIT protein in knockout mice (FHIT−−) results in a loss of tumor formation ability, demonstrating this gene’s role in the process of carcinogenesis and its possible function as a tumor suppressor [17].

At the same time, exon 8, which contains the domain of the histidine triad, is frequently absent in carcinomas, suggesting that this exon has an essential function that is lost in the process of tumorigenesis. In *in vitro* studies, FHIT acts as a typical Ap3A [3]. The triad’s presence is essential to the catalytic activity of FHIT, as a single substitution of the central histidine for asparagine leads to a loss of hydrolytic capacity. Moreover, the altered FHIT protein continues to suppress the formation of tumors, which shows that this suppression is independent of the Ap3A hydrolytic enzymatic activity [18]. Perhaps FHIT functions as an important molecular signal which, by means of Ap3A ligation, determines stops in the cell cycle for repairing DNA damage or induces apoptosis [19, 20].

Changes in the FHIT gene are considered an early event in breast carcinogenesis because they are present from pre-neoplastic lesions to the advanced stages of breast cancer [8, 14, 21]. As in the literature, the present authors observed changes in FHIT in benign breast alterations, in

<table>
<thead>
<tr>
<th>Genotyping</th>
<th>FHIT expression¹</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence of LOH</td>
<td>1 (6.7%)</td>
<td>14 (93.3%)</td>
</tr>
<tr>
<td>Absence of LOH</td>
<td>1 (5.9%)</td>
<td>16 (94.1%)</td>
</tr>
</tbody>
</table>

¹ The category “weak positive” was excluded from the Table since it was not observed in any patient.

in situ carcinoma, and in all the different stages of invasive carcinoma.

Regarding the conventional prognostic factors, the authors also found no significant difference in lymph-node compromise, histological grade, and LOH within the FHIT gene. These results are in agreement with data available in the literature that did not find differences in the rate of loss of heterozygosity in different grades of invasive carcinoma nor in tumors with metastases to axillary lymph nodes [16]. On the other hand, patients with concomitant LOH at BRCA1 e FHIT loci had poor prognostic factors, such as large tumors, axillary nodal involvement, severe histologic grade, peritumoral vascular invasion, and hormone receptor negative status. Likewise, the concomitant LOH at these genes leads to a shortest survival compared with patients without LOH [15].

The life style and demographic variables did not lead to alterations in the locus of the FHIT gene. Otherwise, an interesting fact was observed in the present study: a significant association between menopause and LOH in FHIT. The multivariate analysis showed that women in post-menopausal status present a chance of 5.40 (95% CI = 1.12 - 26.05) times greater to show LOH in the FHIT gene when compared with patients in the premenopausal stage.

Studies report that 80% of breast tumors with genetic changes showed a loss or significant reduction in the expression of the FHIT protein [22]. However, the authors did not find an association between LOH in the FHIT gene and changes in the protein expression. It may be that this protein is expressed, but do not know whether it is functioning, since the presence of intragenic changes was detected.

Furthermore, it has been described that breast cancers with no expression of the FHIT protein showed genetic changes and that 73% of the tumors with decreased protein expression had LOH, demonstrating a relationship between FHIT protein expression and changes in the gene’s locus [23]. Nonetheless, the present authors found one patient with no FHIT protein expression without visible changes in the gene. There may exist changes in other regions not covered by the markers used in this study or even other genetic changes that are undetectable by LOH analysis [24].

In addition to LOH in FHIT, homozygotic deletions have been described in sporadic breast carcinomas and benign breast lumps, with these deletions being responsible for the loss of FHIT protein expression [12]. Other studies suggest that reduced expression of mRNA and
Loss of heterozygosity in the fragile histidine triad (FHIT) locus and expression analysis of FHIT protein in patients etc.

After a long period of tracking patients with breast cancer and analyzing FHIT expression, some authors reported a normal or intermediate protein expression in patients with a good prognosis for breast cancer, and they associated this expression with a higher disease-free survival, while an absence of FHIT was associated with a worsening evolution of these tumors [26].

Although FHIT function has not yet been precisely established, various studies suggest that the gene may be an important target for gene therapy and for drug development in future, since various studies have shown that FHIT is a good marker of prognosis in various types of cancer [17, 23, 27]. In conclusion, the authors observed LOH in 48.6% of women with breast diseases, although the FHIT protein continued to be expressed. Furthermore, changes in the locus of FHIT were observed from pre-malignant lesions through to the advanced stages of breast cancer. In addition, in spite of the majority of demographic, clinical, and related risk factors, variables were not associated with LOH at FHIT gene, women in post-menopausal stage presented a higher chance to show alterations in the FHIT locus. Future studies should aim to explore further associations between FHIT expression and disease outcomes.

Table 5. — Comparison between different clinical, gynecological and demographic variables, and rates of loss of heterozygosity in the FHIT gene in patients with breast disorders.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FHIT (%)</th>
<th>Loss of heterozygosity (LOH)</th>
<th>p value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>8/18 (44.4%)</td>
<td>0.615</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>9/17 (52.9%)</td>
<td>1.41 (0.37-5.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>11/22 (50.0%)</td>
<td>0.826</td>
<td></td>
<td>1.17 (0.29-4.61)</td>
</tr>
<tr>
<td>Black</td>
<td>6/13 (46.2%)</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Menarche</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early (≤ 12 years)</td>
<td>7/17 (41.2%)</td>
<td>0.312</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Normal (13-15 years)</td>
<td>6/12 (50.0%)</td>
<td>1.43 (0.32-6.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late (≥ 16 years)</td>
<td>4/5 (80.0%)</td>
<td>5.71 (0.52-62.66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5/18 (27.8%)</td>
<td>0.006 / 0.036</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>12/16 (75.0%)</td>
<td>7.80 (1.69-36.06) / 5.40 (1.12-26.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First gestation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>3/4 (75.0%)</td>
<td>0.558</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>&lt; 30 years</td>
<td>11/24 (45.8%)</td>
<td>0.28 (0.03-3.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 30 years</td>
<td>3/6 (50.0%)</td>
<td>0.33 (0.02-5.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous abortion episode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8/21 (38.1%)</td>
<td>0.129</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>9/14 (64.3%)</td>
<td>2.93 (0.72-11.91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13/23 (56.5%)</td>
<td>0.621</td>
<td></td>
<td>1.95 (0.43-8.83)</td>
</tr>
<tr>
<td>Yes</td>
<td>4/10 (40.0%)</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Breast disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>4/13 (30.8%)</td>
<td>0.105</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Malignant</td>
<td>13/22 (59.1%)</td>
<td>3.25 (0.76-13.89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15/32 (46.9%)</td>
<td>0.242</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>2/2 (100.0%)</td>
<td>5.65 (0.25-126.88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>9/22 (40.9%)</td>
<td>0.151</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yes</td>
<td>8/12 (66.7%)</td>
<td>2.89 (0.66-12.6)</td>
<td></td>
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<tr>
<td>Contraceptives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13/24 (54.2%)</td>
<td>0.452</td>
<td>1.77 (0.39-7.93)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4/10 (40.0%)</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3/5 (60.0%)</td>
<td>0.879</td>
<td>1.39 (0.20-9.71)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14/27 (51.9%)</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4/8 (50.0%)</td>
<td>0.838</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive</td>
<td>13/24 (54.2%)</td>
<td>1.18 (0.24-5.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIB-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7/11 (63.6%)</td>
<td>0.388</td>
<td>1.93 (0.43-8.61)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10/21 (47.6%)</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

Values highlighted in italics refer to the multivariate logistic regression result, in which only menopause remained in the final model with statistical significance.
to clarify FHIT’s precise mechanism of action in breast epithelials and the involvement of these proteins in the process of tumorigenesis.

Acknowledgements

This work was supported with grants from Fundação de Amparo À Pesquisa do Estado de Minas Gerais (Fapemig) and Conselho Nacional de Pesquisa e Desenvolvimento (CNPq). The authors thank the members of the Parasitology Laboratory (UFTM) for the use of their PCR facilities and IPON (Instituto de Pesquisa em Oncologia), UFTM for providing the samples.

References

The value of negative chlamydia trachomatis antibody in prediction of normal tubes in infertile women

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Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran (Iran)

Summary

Objective: To evaluate the value of Chlamydia trachomatis antibody testing in prediction of at least one normal tube in infertile women. Materials and Methods: Eighty infertile women without any history of abdominal or pelvic surgery, pelvic inflammatory disease, and endometriosis were recruited in this cross-sectional study from 2009 to 2010. The patients underwent hysterosalpingography, laparoscopy, and anti Chlamydia trachomatis IgG antibody (CAT) testing. We compared laparoscopy findings and CAT regarding sensitivity, specificity, accuracy, and predicting value of tubal conditions. Results: The CAT was positive in 50 patients (62.5%) and laparoscopy was positive in 32 patients (40%). The CAT was significantly higher in women with tubal disease (1.88 ± 0.34) versus in women with normal tubes (1.21 ± 0.28) (p = 0.003). Five out of 30 sero-negative women had unilateral tubal abnormality and none of them had bilateral tubal obstruction or severe pelvic adhesion. The sensitivity, specificity, positive and negative predictive value, and accuracy of the CAT in prediction of one normal tube were 100%, 42.25%, 18%, 100%, and 48.75%, respectively. Conclusion: The negative predictive value of CAT to predict at least one normal tube in infertile women without history of abdominal or pelvic surgery, pelvic inflammatory disease, and endometriosis was 100%.

Key words: Chlamydia trachomatis antibody; Fallopian tube evaluation; Female infertility; Predictive value.

Introduction

Tubal factor is one of the most important factors in the diagnosis and treatment of infertile women. The evaluation of tubal factor requires invasive techniques such as hysterosalpingography (HSG) and laparoscopy. Laparoscopy is the gold standard for evaluation of tubal abnormalities but it is expensive, invasive, and requires general anesthesia. Thus, using a noninvasive and cost benefit technique to evaluate tubal factor may reduce unnecessary laparoscopy.

Genital Chlamydia trachomatis infection has a worldwide distribution [1] and is recognized as the single most common cause of tubal peritoneal damage [2, 3]. Previous studies have confirmed a strong correlation between positive Chlamydia serologic results and salpingitis, which results in infertility [4, 5] and the other study revealed that the severity of tubal disease correlates with an increase in antibody titer [6]. Keltz showed that Chlamydia serology as a screen test for tubal infertility is an inexpensive, noninvasive test that matches the predictive value of most standard infertility tests [7]. Because pregnancy is possible with one normal tube, the aim of this study is to evaluate the value of the Chlamydia IgG antibody (CAT) in prediction of at least one normal tube in infertile women.

Material and Methods

In this cross-sectional study we enrolled 80 infertile women referred to the Gynecology clinic in Akbarabadi and Raso- lakram hospitals, Tehran University of Medical Sciences from April to December 2010. This study was approved by the ethical committee of Tehran University of Medical Sciences. Informed consents were given by all participants. Exclusion criteria were history of four previous abdominal or pelvic surgeries, history of pelvic inflammatory disease, history of tuberculosis in patient or her family, history compatible with endometriosis (premenstrual spotting, progressive pelvic pain, dysmenorrhea, dysparunia and pain in defecation), and male factor infertility.

After history taking and general physical examination, infertility workups such as semen analysis, HSG, and hormonal assay were carried out on all patients. Venous blood (5 cc) was drawn for laboratory measurement of the serum CAT. CAT was measured by the Elisa technique (trinity USA). According to the kit brochure, the positive titer was $\geq 1.1$ and the negative titer was $\leq 0.9$. A titer of 0.91-1.09 was considered suspicious. If the patient’s CAT was in this range the test was repeated three weeks later. Laparoscopy was performed for all patients because of history of abnormal HSG, unexplained infertility, and demand for ovarian cauteterization. Laparoscopic findings were categorized as normal, unilateral tubal obstruction or abnormality, bilateral tubal obstruction, and frozen pelvis. All data were analyzed using SPSS. Statistical evaluation was performed using Student’s t-test and chi square tests. Statistical significance was defined as $p < 0.05$ and the results were expressed as means ± SD and percentage. CAT data were compared to laparoscopy findings regarding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and accuracy.

Results

We studied 80 infertile women with a mean age of 26.7 ± 3.8 years and mean duration of infertility of 4.8 ± 2.1 years. Sixty-eight out of the 80 patients (85%) had been treated for at least one cycle for infertility.
CAT was positive in 50 out of 80 patients (62.5%). Evidence of tubal disease which identified by laparoscopy was present in 32 patients (40%) (Table 1).

Seropositive woman had a significantly higher incidence of tubal abnormality in comparison with seronegative women ($p = 0.002$). CAT sensitivity, specificity, PPV, NPV, and accuracy to predict tubal abnormality were 84%, 52%, 54%, 83% and 65%, respectively. CAT levels were significantly higher in women with tubal disease ($1.88 \pm 0.34$) in comparison with the normal tube group ($1.21 \pm 0.28$, $p = 0.003$) (Table 2).

Twenty-three patients in our study had at least one normal tube with unilateral tubal pathology (28.75%) and nine patients had at least two-sided tubal pathology (11.25%). CAT levels were significantly higher in women with bilateral tubal pathology compared to unilateral (2.37 ± 0.42 vs 1.39 ± 0.27) ($p < 0.0001$). Three out of nine patients with bilateral tubal damage had frozen pelvis. The mean of CAT in this group was 2.8 ± 0.54 and in women with bilateral tubal pathology without frozen pelvis it was 2.2 ± 0.89 ($p = 0.001$).

One normal tube was identified by laparoscopy in all patients with a negative CAT (Table 3). The CAT sensitivity, specificity, PPV, NPV, and accuracy for prediction of at least one normal tube were 100, 42.25, 18, 100, and 48.75, respectively.

We also compared the HSG findings to laparoscopic findings (Table 4) and the predictive values of HSG in comparison with CAT are shown in Table 5.

**Discussion**

The most common sexually transmitted disease is Chlamydia trachomatis and CAT, as an inexpensive and non-invasive screening test for tubal factor infertility, has been presented in the fertility work-up. However, laparoscopy is considered the gold standard for the evaluation of tubal function which is an invasive and expensive procedure requiring general anesthesia.

In this study we evaluated the value of the negative CAT in prediction of at least one normal tube in infertile women. Our study showed CAT has a sensitivity and NPV of about 100% to predict at least one normal tube. This could be important for the inconvenience which many women face for tubal testing such as laparoscopy. CAT was negative in five patients who had tubal abnormalities in laparoscopy (17% false negative rate). All of these five women had unilateral tubal abnormalities and one normal tube, indicating that CAT can predict at least one normal tube with sensitivity and NPV of 100%.

Pregnancy is possible with one normal tube. So we can offer measurement of antichlamydia antibody as screening test at starting of evaluation of infertile couples that leads to prevention of invasive techniques as laparoscopy. However, there are challenges in our conclusion about negative CAT. The first one is the other pathologic micro organisms which would be responsible for tubal disease. According to Vlasak study, PID with organisms such as anaerobes or facultative aerobes may be initiated by gonorrhea, Chlamydia or both [8] and the second one is this fact that not all women develop Chlamydia trachomatis antibody after a Chlamydia infection [9, 10]. Time-related antibody titer decline is also a possible reason for false negative results.

In these cases, some studies have suggested a chronological decline in titer [11, 12] but Gijsen et al. in their study revealed C. trachomatis IgG antibodies never became undetectable [13]. Rodgers et al. also showed that there is a novel link of tubal factor infertility with Chlamydial anti-clpp. (caseinolytic protease protein) antibody [14].

HSG is a less invasive test, as well to assessment of tubal patency is associated with a post procedure pregnancy rate from 12% to 33% [15]. In our study HSG
showed a high specificity and low sensitivity. Thus if HSG and CAT are combined, it can be suggested that laparoscopy can be omitted in a normal CAT and also in a very high level of CAT. In high titers of IgG (at least two times greater than normal), it can predict bilateral tubal occlusions and thus it may reduce many unnecessary laparoscopy procedures. In our study high titers of CAT were seen in the nine patients who had frozen pelvis or bilateral tubal obstructions. There were no false positive results in these nine patients. Thus laparoscopy can be avoided and assisted reproductive technique can be directly offered to these infertile women.

The limitations of the current study were small sample size, lack of a control group, and infection because of other microorganisms. In this case, to achieve better and comparable results, we suggest a case control study with large sample size and close attention to other infections in infertile women.

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Evaluation of low-dose letrozole addition to ovulation induction in IVF

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Summary

Purpose: The aim was to investigate the impact of low-dose letrozole usage along with gonadotropin treatment in vitro fertilization (IVF) cycles in comparison to gonadotropin treatment alone. Materials and Methods: Fifty patients were prospectively included in this randomized study and were divided into two groups. Age, demographic features, causes, and period of infertility were adjusted and matched for both groups. Group 1 included 25 patients who received gonadotropin treatment and letrozole along with gonadotropin-releasing hormone (GnRH) antagonist protocol; group 2 included 25 patients who received gonadotropin treatment along with GnRH antagonist protocol. Results: Total follicle-stimulating hormone (FSH) and daily FSH doses were lower in group 1, although not statistically significant (p > 0.05). The period of ovulation induction was significantly shorter in group 2. While numbers of retrieved oocytes and transferred embryos were lower in group 1, they were not statistically significant (p > 0.05). Number of clinical pregnancies per embryo transfer, number of clinical pregnancies per cycle, and number of ongoing pregnancies (> 16 gestational weeks) were similar in both groups (p > 0.05). Conclusions: Addition of low-dose letrozole to gonadotropin treatment in GnRH antagonist protocols may result in a lower dose of gonadotropin administration. However, routine clinical practice remains questionable due to no evident positive effect on pregnancy rates.

Key words: Aromatase inhibitor; Gonadotropin; GnRH antagonist; In vitro fertilization; Letrozole; Ovulation induction.

Introduction

Letrozole is an aromatase inhibitor (AI) that was first used in the treatment of advanced breast cancer in 1997 [1]. Then, it was soon realized that letrozole had potential for the treatment of other estrogen-dependent conditions, particularly in the field of gynecology. One application area of interest is the use of letrozole for induction of ovulation in women with World Health Organization (WHO) type II anovulation. The first report in this area was by Mitwally and Casper [2]. They argued that letrozole was effective in inducing ovulation in women with polycystic ovarian syndrome (PCOS). The use of letrozole for superovulation prior to in vitro fertilization (IVF) was also explored, especially its usage in women who had responded poorly to conventional treatment with gonadotropins [3-5]. Recently, some studies showed that addition of letrozole in IVF treatment may reduce the amount of gonadotropin administration that is required for ovulation induction [3, 6-7]. Less gonadotropin exposure may have a positive impact on oocyte quality and consequently pregnancy rates. In addition, reduced gonadotropin administration may have a beneficial effect on treatment cost.

The aim of this study was to investigate the impact of low-dose letrozole usage along with gonadotropin treatment in IVF cycles, in comparison to gonadotropin treatment alone, and the subsequent effect on IVF outcome.

Materials and Methods

Fifty patients administered to the Infertility Clinic of Istanbul University School of Medicine were prospectively included in this randomized study. Approval of the ethics committee and informed consent from all participants were obtained prior to the treatment.

Patients were divided into two groups. Age, demographic features, causes, and period of infertility were adjusted and matched for both groups. Group 1 included 25 patients who received gonadotropin treatment and letrozole along with gonadotropin-releasing hormone (GnRH) antagonist protocol, while group 2 included 25 patients who received gonadotropin treatment along with GnRH antagonist protocol without letrozole during IVF.

All patients included in the study received a thorough physical examination and were assessed with transvaginal ultrasoundography (TVUS). Patients who were clinically infertile for at least two years and who were attempting IVF for the first time were included in the study. Reasons for infertility were male factor, ovarian factor, tubal-peritoneal factor, or unexplained factor. Male factor infertility was evaluated with semenogram that was repeated twice in which WHO global reference values for human semen characteristics were utilized. Hysterosalpingography was also used as a diagnostic tool to identify potential tubal and peritoneal pathology in all patients. Patients were assessed by day-3 follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) levels as well. Thyroid function test and prolactin values were reviewed and patients who had lower or higher than normal results were excluded from the study. TVUS was performed on the day-2 of the menstrual cycle and patients who had ovarian cysts were also excluded from the study.

Exclusion criteria were: age above 40 years, FSH levels of more than 15 IU/l, antral follicle count (AFC) less than 5, body mass index (BMI) greater than 30, any abnormal ultrasound results (i.e. cyst, endometrioma, endometrial polyp, etc.), and previous IVF attempt(s).

Either Puregon® (Schering-Plough, NJ, USA) or Gonafol® (EMD Serono, MA, USA) was used in controlled ovarian stimulation (COH) which was first administered on day-3 of the
menstrual cycle. Initial gonadotropin dosage was decided according to the patient’s age, AFC, BMI, FSH, E2, and ovarian reserve, and was regulated with a range from 150 IU to 225 IU. It was then adjusted according to the response of ovarian follicles, which were followed-up via TVUS. The total dosage administered to every patient was recorded. In addition, patients in group 1 also received 2.5 mg letrozole orally starting on the day-2 of the menstrual cycle and continued the usage until day 6. In both groups, when the dominant follicle size was ≥ 16 mm, GnRH antagonist protocol was initiated by administration of 0.25 mg cetrorelix acetate, which was continued until the human chorionic gonadotropin (hCG) injection day. Ovarian follicular development was observed via TVUS at a one- to three-day frequency. When at least three follicles ≥ 16 mm in size were found, 10,000 IU hCG were injected to achieve follicular maturation. Oocyte retrieval (OCT) took place 36 hours after hCG administration. The number of retrieved MII oocytes was recorded. The first morning-after oocyte retrieval, all follicles ≥ 14 mm were punctured to recover oocytes. Two hours after puncturing the follicles, all retrieved oocytes were transferred to the uterine cavity according to number of good quality oocytes. Three days after retrieval, one to three embryos were transferred to the uterine cavity via TVUS. The total dosage administered to every patient was recorded. In addition, patients in group 1 also received 2.5 mg letrozole orally starting on the day-2 of the menstrual cycle and continued until day 6. In both groups, when the dominant follicle size was ≥ 16 mm, GnRH antagonist protocol was initiated by administration of 0.25 mg cetrorelix acetate, which was continued until the human chorionic gonadotropin (hCG) injection day. Ovarian follicular development was observed via TVUS at a one- to three-day frequency. When at least three follicles ≥ 16 mm in size were found, 10,000 IU hCG were injected to achieve follicular maturation. Oocyte retrieval (OCT) took place 36 hours after hCG injection. All follicles ≥ 14 mm in size were retrieved. The number of retrieved MII oocytes was recorded. Three days after retrieval, one to three embryos were transferred to the uterine cavity according to number of good quality embryos (grade 1). The number of transferred grade 1 embryos was recorded. The first morning-after oocyte retrieval, all patients received 3 x 200 mg micronized progesterone vaginally as luteal phase support. If pregnancy occurred, vaginal luteal phase support continued until the 12th week of gestation. Twelve days after embryo transfer, β-hCG level in the blood was measured and recorded. If β-hCG level was > 5 mIU/ml in either measurement, it was considered positive β-hCG and patients with such levels were considered as pregnant. At the sixth week of gestation, continuation of pregnancy was confirmed by TVUS. Any complications that occurred during the entire process were recorded.

All statistical calculations were performed using the Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA). After using the Kolmogorov-Smirnov’s distribution (KS) test to examine the normality of data, the significance of the difference between the two compared groups was assessed using Student’s t-test and Mann-Whitney U-test, while non-parametric variables were evaluated by the Mann-Whitney U-test. A p < 0.05 was considered statistically significant.

Results

Demographics of the study population are presented in Table 1. The mean age of patients, etiology of infertility, basal FSH, and E2 concentrations were matched for both groups.

When gonadotropin treatment was compared, total FSH (2,668 ± 1,027.11 vs 2,919 ± 1,126.02) and daily FSH (203.35 ± 101.43 vs 245.29 ± 142.61) doses were lower in group 1, although not statistically significant (p > 0.05). The period of ovulation induction was significantly shorter in group 2 (13.12 ± 2.02 vs 11.9 ± 2.03; p < 0.05) (Table 2). The mean number of follicles > 16 mm (5.28 ± 4.48 vs 4.76 ± 3.32) and endometrial thickness (8.89 ± 1.50 vs 9.45 ± 2.05) on the day of hCG administration were similar in both groups (p > 0.05) (Table 2). However, the mean concentration of serum E2 on the day of hCG administration was significantly higher in group 2 than in group 1 (1,666 ± 337.34 vs 2,848 ± 623.47; p < 0.001) (Table 2).

Table 1. — Baseline characteristics of the patient groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 25)</th>
<th>Group 2 (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age, years</td>
<td>32.6 ± 5.97</td>
<td>32.5 ± 5.01</td>
<td>0.96</td>
</tr>
<tr>
<td>Causes of Infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td>10 (40%)</td>
<td>10 (40%)</td>
<td></td>
</tr>
<tr>
<td>Ovarian factor</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Tuboperitoneal factor</td>
<td>6 (24%)</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>Male factor</td>
<td>7 (28%)</td>
<td>8 (32%)</td>
<td></td>
</tr>
<tr>
<td>Basal FSH (mIU/ml)</td>
<td>8.03 ± 4.20</td>
<td>6.24 ± 3.61</td>
<td>0.11</td>
</tr>
<tr>
<td>Basal E2 (pg/ml)</td>
<td>43.41 ± 25.58</td>
<td>49.79 ± 42.38</td>
<td>0.52</td>
</tr>
<tr>
<td>Infertility period, years</td>
<td>4.44 ± 2.20</td>
<td>5 ± 2.14</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD unless otherwise indicated. NS = not significant.

Table 2. — Response to ovulation induction.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 25)</th>
<th>Group 2 (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dose of FSH used, IU</td>
<td>2,668 ± 1,027.11</td>
<td>2,919 ± 1,126.02</td>
<td>0.41</td>
</tr>
<tr>
<td>Daily dose of FSH used, IU</td>
<td>203.35 ± 101.43</td>
<td>245.29 ± 142.61</td>
<td>0.23</td>
</tr>
<tr>
<td>Duration of induction with FSH, days</td>
<td>13.12 ± 2.02</td>
<td>11.9 ± 2.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Number of follicles &gt; 16 mm on the day of hCG administration, mm</td>
<td>5.28 ± 4.48</td>
<td>4.76 ± 3.32</td>
<td>0.64</td>
</tr>
<tr>
<td>E2 level on the day of hCG administration, pg/ml</td>
<td>1,666 ± 337.34</td>
<td>2,848 ± 623.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Endometrial thickness on the day of hCG administration, mm</td>
<td>8.89 ± 1.50</td>
<td>9.45 ± 2.05</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD unless otherwise indicated.

Table 3. — Embryological data and pregnancy outcome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 25)</th>
<th>Group 2 (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of retrieved oocytes</td>
<td>10.44 ± 6.12</td>
<td>8.76 ± 7.35</td>
<td>0.38</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
<td>1.92 ± 1.11</td>
<td>2.44 ± 0.91</td>
<td>0.07</td>
</tr>
<tr>
<td>Number of clinical transfers per embryo transfer, also in %</td>
<td>7 (31%)</td>
<td>7 (30%)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of clinical transfers per cycle attempt, also in %</td>
<td>7 (28%)</td>
<td>7 (28%)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of ongoing pregnancy (&gt; 16 weeks), also in %</td>
<td>5 (20%)</td>
<td>5 (20%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD unless otherwise indicated. NS = not significant.

Discussion

In this study, the authors sought to determine whether the use of low-dose letrozole in ovulation induction along with gonadotropin treatment could have a positive impact on IVF cycles and subsequently, clinical pregnancy rates. Goswami et al. carried out the first randomized clinical trial (RCT) with the use of letrozole in IVF treatment cycles published in 2004 [3]. The study consisted of 38
patients, and showed a significantly lower dosage of total FSH in the letrozole group with a comparable pregnancy rate. Oktay et al. in a randomized study found a decrease of 44% in the total FSH dosage again in the letrozole group [6]. A RCT by Ozmen et al. also confirmed the use of a lower dose of FSH in the letrozole group [5]. An observational study, consisting of 147 poor-responding patients, of Garcia-Velasco et al. revealed a better implantation rate in letrozole group but no significant difference in the total FSH dosage [4]. The findings in this study revealed a lower total FSH dosage in the letrozole group, although the difference was not statistically significant.

Prospective trial of Schoolcraft et al. did not find a difference in the ovulation induction period between the two groups in a prospective study consisting of 534 patients [7]. A retrospective case-controlled study of Yarali et al., which consisted of 885 patients, revealed a shorter period of ovulation induction in the letrozole group [8]. In contrast, the authors found that the period of ovulation induction was significantly longer in the letrozole group. On the other hand, although statistically insignificant, total and daily FSH doses were lower, while number of follicles was higher. Therefore, it can be argued that low-dose letrozole may have a positive impact on the initial period of ovulation induction when follicles mature.

Verpoest et al. carried out a prospective study with a small study group, consisting of 20 patients [9]. Their results indicated an increased number of retrieved oocytes in the letrozole group that was not statistically significant.

Schoolcraft et al. showed a non-significant increase in the ongoing pregnancy rate in the letrozole group [7], whereas the study of Yarali et al. revealed a significantly higher implantation rate in the letrozole group [8]. Ozmen et al. did not find a statistically significant difference in pregnancy rate per cycle, pregnancy rate per transfer, and ongoing pregnancy rate between the two groups [5]. A study by Lee et al., consisting of 53 patients who were poor responders, revealed comparable live birth rates [10]. In a very recent study, Miller et al. evaluated the impact of letrozole usage in patients with suspected endometrial receptivity defects [11]. They concluded that the usage of letrozole resulted in higher conception rates in patients with endometrial receptivity defects. Our findings revealed no significant differences between the groups in regard to clinical pregnancy.

Fouda and Sayed carried out a RCT to compare the low-dose letrozole regimen (2.5 mg/day) to the high dose letrozole regimen (5 mg/day) [12]. They found no significant differences between the two groups with regards to the number of oocytes retrieved and clinical pregnancy rate. The authors opted for low-dose letrozole regimen (of 2.5 mg/day from cycle days 2-6 in this study.

**Conclusion**

The addition of low-dose letrozole to gonadotropin treatment in GnRH antagonist protocols may result in a lower dose of gonadotropin administration. However, routine clinical practice remains questionable due to no evident positive effect on pregnancy rates. Consequently, further research in carefully controlled clinical trials is needed to determine whether higher-dose letrozole may be effective and whether the benefit is worth the cost of the treatment.

**References**


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Lentivirus vectors mediated eGFP transfected into rat ovary in vivo

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Summary

Purpose: To evaluate the optimum dosage and time-effect relationship of lentivirus vectors mediated enhanced green fluorescence protein gene (lenti-eGFP) transfection into the rat ovary in vivo. Materials and Methods: Lenti-eGFP was microinjected into rats’ ovaries with different dosage (2 × 10^6 TU and 10 × 10^6 TU virosome respectively, n = 5 rats). The expression of eGFP was examined by the fluorescence microscope at injection at five days. The fluorescence intensity of different dose groups was calculated and determined the optimum dosage. The authors observed the expression of eGFP in ovaries and other tissues at days 5, 15, 30, 45, 60, and 75 after the rats’ ovaries were microinjected with the optimum dosage of lenti-eGFP. Reserve transcription-polymerase chain reaction (RT-PCR) and RT-quantitative (q) PCR (RT-qPCR) were used to qualitative and quantitative analyze the expression of eGFP in different tissues and organs of transfected rats. Results: The expression of eGFP in both ovaries of every rat was seen at five days of transfection. Semi-quantitative assessment of green fluorescence for the two-dosage group was 0.2311 ± 0.0203 and 0.2307 ± 0.0199, respectively. There was no significant difference in both groups (p = 0.976). The expression of eGFP enhanced with transfection time prolongation and continued with 75 days of transfection (the fluorescence density in different time was 0.2307 ± 0.0199, 0.3119 ± 0.0213, 0.3462 ± 0.0264, 0.3568 ± 0.0127, 0.3496 ± 0.0133, and 0.3513 ± 0.0172, respectively). Furthermore, there were efficient and durable expressions of eGFP in other tissues and organs of rats. RT-PCR and RT-qPCR proved these results. Conclusion: Lenti-eGFP may successfully transflect ovary tissues and other organs in vivo simultaneously, the expression of eGFP is highly efficient and durable.

Key words: Lentivirus vectors; Enhanced green fluorescence protein; Ovary; In vivo; Animal experiment.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder. The incidence is 4%-12% [1-4]. In some areas of China, the epidemiological survey showed its incidence rate to be 6%-8% [5, 6]. It is one of the most common causes of sterility in young women. The pathogenesis for the disease is not clear. Environment and inheritance (gene mutation) working together is accepted by most of researchers. There is yet no satisfactory effect in clinical treatment. There could be a breakthrough in its treatment through an appropriate intervention of the mutant gene by transgenic method in concomitance with lifestyle change (diet and exercise). Hence, the authors attempted to explore a transgenic treatment for PCOS. There are less researches regarding gene transfection into ovary in vivo. In the present study, the authors used lentivirus vectors mediated enhanced green fluorescence protein (eGFP) gene to transfect rat ovary in order to verify its effectiveness.

Materials and Methods

Animals

All animal experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee at Chongqing Medical University in Chongqing city (China). Forty specific pathogen-free (SPF) degree Sprague-Dawley female rats two months of age (weighing 160 to 180 g) were provided by Animal Center of Chongqing Medical University and housed in a 12-hour light/dark cycle at a controlled temperature and humidity with free access to food and water.

Rats were randomly assigned to the following eight groups: control, low-dose, high-dose, 10 days, 15 days, 30 days, 45 days, 60 days, and 75 days (n = 5, respectively). At five days of transfection, the optimum dosage was to be selected between the two doses by infection efficiency observed with a fluorescence microscope.

Lenti-eGFP transfection into rat ovaries and microinjection

The animals were fasted overnight but were allowed free access to water. The body weight for each rat was calculated prior to surgery. Anesthesia was induced with 10% chloral hydrate at a dose of 100 mg/kg by intraperitoneal injection. Rats were subjected to the prone position and a microtubule nick about 1.0 - 1.5 cm at right back of body surface projection for ovary. Hypodermal, muscular layer, and peritoneum were cut and the ovary was drawn out from abdominal cavity.

Different dosage viral particle of lenti-eGFP was microinjected into sub-envelope of ovary. The low-dose group was 2 × 10^6 TU and high-dose group was 10 × 10^6 TU virosome every rat respectively. The surgery of microinjection is shown in Figure 1.

The expression of eGFP

Frozen and paraffin-imbedding sections were prepared to fluorescence microscope for each group of rat ovaries. The green fluorescence density for ovary sections of every group was calculated and the statistic difference was analyzed.

RT-PCR and real time quantitative PCR

Total RNA was extracted from ovary tissues stored at -80°C using the RNAiso plus extraction method as directed. Briefly, total RNA was extracted with RNAiso plus and precipitated with isopropyl alcohol, washed in ethanol, and re-suspended in

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RNase-free water. RNA quantity and quality were determined by spectrophotometry. Two micrograms of total RNA were used for reverse transcription (RT) and the RT reagent kit method as described. CDNA was used for polymerase chain reaction (PCR) and quantitative PCR (qPCR) and repeated three times. PCR and a qPCR products were synthesized. Each 20 μl SYBR Green reaction contained 1.0 l cDNA, 0.1 mol/l: forward primer 0.75 μl and 0.1 μmol/l reverse primer 0.75 μl. For amplification of both eGFP and the reference gene actin-beta, the following PCR protocol was applied: 95°C for 60 s, 95°C for 5 s, 55°C for 30 s, 40 cycles. The specific primers for qPCR of eGFP gene were F1 5’-ctttcggttatggtgttcaatg-3’, R1 5’-tgtcttgtagttcccgtcatctt-3’, and product size was 137 bps; the specific primers for actin-beta gene were F1 5’-caccceggtagtaacctc-3’, R1 5’-ccacccccaccaacaccc-3’, and product size was 207 bps. The fluorescence spectra were recorded during the elongation phase of each PCR cycle. The results were analyzed by the delta Ct (ΔCt) method, which reflects the difference in threshold for the target gene relative to that of actin-beta in each sample. The specific primers for PCR of eGFP were F1 5’-gcgagggcgatgccacctac-3’, R1 5’-cggttcaccagggtatctcc-3’, and product size was 267 bps.

Data analysis

IPP 6.0 software was used to calculate the fluorescence density of ovary tissue sections for every group. Data were presented as means ± Std. deviation. SPSS 16.0 was used to all data statistics. Statistical differences among the various groups were assessed by one-way ANOVA. A value of p < 0.05 was considered statistically significant.

Results

Green fluorescence in the rats’ ovary cortex, stroma, and corpus luteum cells was stronger and was less in complete follicles of different developmental stages at five days of transfection. There was no significant difference for the fluorescence density between the two dose groups. The lower dosage (2 × 10⁶ TU) resulted in a better transfection effect. The expression of eGFP increased as the extension of transfection time reached its peak at 30 days. The green fluorescence was still seen in the ovary at 75 days of transfection (Figure 2). Semi-quantitative outcome for fluorescence density according to different time points is shown in Table 1 and Figure 3. The eGFP expression was not only in the side ovary of virosome injection but also in other tissues and organs. The authors observed the green fluorescence at 30 days of transfection in homolateral fallopian tube, contralateral ovary and fallopian, uterus, brain, cornea, retina, liver, muscle, lung, heart, and fat tissue (Figure 4).
The outcome of RT-PCR (Figure 5) was in accordance with the information depicted in the fluorescence photograph, in which there is no expression of eGFP mRNA in control rats’ ovary but in transfected rats’ various organs and tissues.

RT-qPCR was prepared to the relative quantity of expression eGFP mRNA. Different expression levels in transfected rat ovaries and other organs and tissues are shown in Figure 6.

**Discussion**

The conception of transgenic treatment began in the middle of the 20th century. It was praised as a revolutionary technology for those diseases of basic molecular level abnormality. It mainly aims to carry the exogenous therapeutic genes into target cells with specific vectors. The gene makes a target treatment through correction of mutation sequences of pathogenic gene or reprogramming host cell function [7, 8]. The lentivirus vectors basic of HIV-1 render the target gene expression stabilized and durable because they may integrate the gene into host cell chromosomes. Several researches indicate the reorganized vector has no cytotoxicity for animals in vivo [9, 10].

Currently, the gene therapy animal models basic of lentivirus vectors includes various cancers and some metabolic diseases [11-14]. Even the foreign gene can be targeted and expressed into specific cells and tissues [15] and it is also developing the direction for future gene therapy.

There are less reports regarding ovarian-related disease gene transfection in vivo. In the experiment the authors used lentivirus vectors mediated report gene-eGFP to observe its expression in rat ovaries and other organs in vivo through ovary-microinjection.

Transfection efficiency is related to different cells and tissues [16, 17]. The larger the carried gene, the lower the transfection efficiency will be. In this experiment, the same fluorescence density in the two different dose virosome ovaries was seen. The authors believe that the lower dosage is adequate for rat ovary.

<table>
<thead>
<tr>
<th>Table 1. — Statistics of semi-quantitative eGFP expression in rat ovaries at different time intervals.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 5</td>
</tr>
<tr>
<td>Mean density</td>
</tr>
<tr>
<td>Std. deviation</td>
</tr>
<tr>
<td>p value</td>
</tr>
</tbody>
</table>

The p value is an outcome compared to the previous time interval.
They observed on transfection five days that eGFP was highly and efficiently expressed in several ovarian cells and tissues including epithelial cells, thecal cells, granular cells, and ovarian medulla.

An interesting phenomenon found is that eGFP expression is less in complete follicles but more in corpus luteum. An essential factor for successful transfection is direct cell-to-cell contact. So the authors presume the complete follicular walls could have a barrier function to delay the virosome into follicles and the barrier could be broken when the follicle ruptures to develop corpus luteum. There is no apparent difference between follicles and corpus luteum for eGFP expression at 15 days of transfection.

During the time-effect observation of eGFP expression, the authors found the expression of eGFP was at peak and durable at 30 days transfection. There is no significant difference for the fluorescence density in ovaries between at 30 and 75 days transfection. The authors suppose lentivirus vectors mediated exogenous genes could
express at long-term and in a stable form in ovarian tissues in vivo. They also discovered eGFP expression in other several organs and tissues including brain and eye. An ideal transgenic therapy would be to target transfection into specific cells [15], but the current vectors are unsuccessful in this. Nonetheless, the non-choice transfection could play an important role in treatment of several genetic and endocrine and metabolic diseases such as PCOS. The authors now believe that the cause of PCOS is the “co-operation” of several genes [18]. Gene mutation occurred not only in the ovary but in other tissues, namely non-choice mutation. The non-specific transfection is just for the non-specific mutation.

Moreover, the authors created a new method for rat ovary microinjection. Traditionally, abdominal incision serves as a belly-cavity operation. There are two main shortcomings for ovarian microinjection. The rat ovaries are located in deep abdominal cavity and near posterior peritoneum, so it is difficult to withdraw them. Furthermore, the abdominal incision must be large enough to complete the operation. However, these problems can be resolved if the incision is selected on the back of the rats where the surface projection of the ovary is located. The advantages for this include less incision, less lesion, and facilitated ovary exposure.

In a conclusion, lentivirus vectors mediated gene could express stable in a form at long-term in rat ovary.

Acknowledgement

The authors thank Professors Chen Lixue and Tang Weixue from the Department of Neurology laboratory in the First affiliated Hospital of Chongqing Medical University for their skillful assistance and technical advice.

References


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Practical biometric ratios of first-trimester screening

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³Istanbul University Cerrahpasa Medical Faculty, Department of Radiology, Istanbul (Turkey)

Summary

Purpose: The authors aimed to determine some practical contributive biometry ratios of the first trimester screening in order to note more accurate measurements and recognize abnormal/mistaken measurements. Materials and Methods: All medical records of singleton pregnancies whose first-trimester screening that was performed between the years of 2004-2010, were evaluated retrospectively. Singleton pregnancies with detected/suspicious anatomical or genetic fetal anomalies, any systemic disease, and familial genetic diseases were excluded. The following ratios were calculated and compared: measurements of biparietal diameter [BPD], head circumference [HC], abdominal circumference [AC], femur length [FL], and crown rump length [CRL] of included fetuses, to each other were calculated. Mean and standard deviations of the ratios were determined for each gestational weeks of 110-6, 120-6, and 130-6. Results: A total of 1,615 singleton pregnancies were included in the data analyses according to exclusion and inclusion criteria. Mean maternal age was 29.5 ± 4.6 years. Mean gestational age of the fetuses was 12.6 ± 0.6 weeks. Mean and standard deviation of the ratios were as follows; CRL/BPD: 3.0 ± 0.2; AC/BPD: 3.0 ± 0.2; CRL/AC: 1.0 ± 0.1; CRL/HC: 0.8 ± 0.1; CRL/FL: 8.8 ± 1.6; BPD/FL: 2.9 ± 0.6; AC/FL: 8.9 ± 1.6; HC/FL: 11.1 ± 2.2, and HC/AC: 1.3 ± 0.1. Among these ratios the standard deviation was small in the ratios of CRL/BPD, AC/BPD, CRL/AC, HC/FL, CRL/HC, and HC/AC. The equations of these ratios were derived from linear regression analyses. The AC/BPD, and CRL/AC ratios had lower R² values than others, indicating a rather constant ratio. Conclusions: The ratios of CRL/BPD, AC/BPD, and CRL/AC seem more practical to be used in the first-trimester fetal ultrasonography practice.

Key words: Practical; Ratio; Screening; First Trimester; Pregnancy; Ultrasonography; Fetus.

Introduction

Within the two recent decades, ultrasonography gained an important place in first-trimester screening and much information has been gained regarding fetal sonography related to normal and abnormal fetuses [1]. There have been many nomograms constructed for everyday clinical practice. However, expertise is still mandatory for the evaluation of many aspects of fetal sonography [2]. Therefore, some practical ratios, if present uniformly, might be helpful for sonography trainees as well as experts to recognize mistaken or abnormal measurements during their trainings.

In this study the authors aimed to determine some practical contributive biometry ratios related to first-trimester screening of singleton pregnancies using all parameters gained in these examinations.

Materials and Methods

All medical records of singleton pregnancies screened between 2004 and 2010 were analyzed retrospectively. Data of singleton pregnancies who were screened at 110-136 gestational weeks, was used for study analyses. Singleton pregnancies with any detected/suspicious anatomical or genetic fetal anomalies, maternal systemic disease, and familial genetic diseases were excluded.

First-trimester screenings were performed accordingly to the criteria previously reported in the literature [3]. Data of the measurements of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), femur length (FL), and crown rump length (CRL) of included fetuses, gestational age, and maternal age were used for statistical analyses. The ratios CRL/BPD, AC/BPD, CRL/AC, HC/AC, CRL/HC, CRL/FL, BPD/FL, AC/FL, HC/FL, BPD/HL, and AC/HL were calculated for each fetus and their percentiles, mean values, and standard deviations were calculated and compared according to each gestational week of 110-6, 120-6, and 130-6. Comparative ratios according to gestational weeks were performed with the Student’s t test. The equations of the graphs of gestational week vs biometric ratios were determined by linear regression analyses. The statistical significance was set as p < 0.05. Statistical analyses were performed using the SPSS version 17.0.

Results

A total of 1,615 singleton pregnancies were included in the data analyses according to exclusion and inclusion criteria. The mean maternal age was 29.5 ± 4.6 years and mean gestational age of the fetuses was 12.6 ± 0.6 weeks. The mean values are shown in Table 1.

Among these ratios, the standard deviation was small in the ratios of CRL/BPD, AC/BPD, CRL/AC, HC/AC, CRL/HC, and HC/AC. The equations of these ratios derived from the linear regression analyses are shown in Table 2. The AC/BPD and CRL/AC ratios had lower R² values than others, indicating a rather constant ratio. Linear regression graphics are shown in Figures 1 and 2.

Comparisons between fetal sexes showed no differences in all of the ratios. When the fetuses were compared according to maternal age (< 35 and ≥ 35 years),
Practical biometric ratios of first-trimester screening

This present study discovered some practical ratios for the first time as compared to those found in the literature. In Table 3, the biometric ratios calculated in the previous studies in the literature are compared with the present study [4-6].

Discussion

The authors have clarified and learned much more regarding prenatal life and its dynamics with the help of ultrasonography. Yet, there still seems more to be clarified and that is why many ongoing enhancements related to sonography techniques are attempted and earlier screening strategies aim to rule out fetal abnormalities with extremely low false positive and negative rates.

Nomograms derived from normative values related to these previous observations have assisted and are still used in daily practice. On the other hand, sonography is an area of expertise used in prenatal screening [2]. In this sense, some practical biometry ratios related to prenatal screening might be helpful as a feedback during sonography. In the current study seeking practical ratios, the authors found that CRL/BPD, AC/BPD, CRL/AC, CRL/H, and HC/AC ratios might be helpful with their lower standard deviation and lower R² values only in AC/BPD and CRL/AC ratios. However, the easiest ones to keep in mind appear to be CRL/BPD and AC/BPD which are approximating three and CRL/AC which is approximating one. This present study discovered some practical ratios for the first time as compared to those found in the literature. In Table 3, the biometric ratios calculated in the previous studies in the literature are compared with the present study [4-6].

Table 1. — Biometric ratios according to gestational weeks.

<table>
<thead>
<tr>
<th></th>
<th>CRL/BPD</th>
<th>AC/BPD</th>
<th>CRL/AC</th>
<th>HC/AC</th>
<th>CRL/FL</th>
<th>FL/BPD</th>
<th>AC/FL</th>
<th>FL/H</th>
<th>HC/FL</th>
<th>BPD/HL</th>
<th>AC/HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.0</td>
<td>3.0</td>
<td>1.0</td>
<td>1.3</td>
<td>0.8</td>
<td>9.9</td>
<td>3.4</td>
<td>10.1</td>
<td>12.9</td>
<td>2.9</td>
<td>8.7</td>
</tr>
<tr>
<td>Std. deviation</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>1.6</td>
<td>0.6</td>
<td>1.6</td>
<td>2.2</td>
<td>0.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Percentile</td>
<td>5</td>
<td>2.6</td>
<td>2.7</td>
<td>0.9</td>
<td>1.1</td>
<td>0.7</td>
<td>7.6</td>
<td>2.5</td>
<td>7.8</td>
<td>9.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.0</td>
<td>3.0</td>
<td>1.0</td>
<td>1.3</td>
<td>0.8</td>
<td>9.7</td>
<td>3.3</td>
<td>10.0</td>
<td>12.7</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>3.3</td>
<td>3.4</td>
<td>1.1</td>
<td>1.4</td>
<td>0.9</td>
<td>12.6</td>
<td>4.3</td>
<td>13.0</td>
<td>16.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Table 2. — Equations of the graphs of gestational week vs biometric ratios.

<table>
<thead>
<tr>
<th>Gestational week</th>
<th>Constant</th>
<th>R²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL/BPD</td>
<td>0.71</td>
<td>0.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AC/BPD</td>
<td>0.014</td>
<td>0.001</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>HC/AC</td>
<td>0.0249</td>
<td>1.564</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRL/AC</td>
<td>0.019</td>
<td>0.75</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRL/H</td>
<td>0.031</td>
<td>0.40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BPD/FL</td>
<td>-0.51</td>
<td>9.39</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRL/FL</td>
<td>-1.32</td>
<td>25.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AC/FL</td>
<td>-1.500</td>
<td>27.657</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HC/FL</td>
<td>-2.136</td>
<td>37.847</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BPD/HL</td>
<td>-0.393</td>
<td>7.468</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AC/HL</td>
<td>-1.233</td>
<td>23.304</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

only the BPD/FL ratio was higher in the younger maternal age group (2.95 ± 0.56 vs 2.86 ± 0.54; p = 0.042). Furthermore, placental sites of the fetuses had no impact on the ratios.

Figure 1. — Linear regression graphic of the CRL/AC ratio.
Figure 2. — Linear regression graphic of the AC/BPD ratio.
Kustermann et al. showed that HC/AC did not show any significant variation with gestational age or CRL [7]. In the study of von Kaisenberg and the present study, this ratio was constant with gestational age as well [4].

In the study of the Johnsen et al., maternal age, fetal sex, and cephalic index influenced the FL/BPD ratio, whereas only fetal sex influenced FL/HC [6]. In the present study, the authors also found no effect of maternal age (except BPD/FL), fetal sex, and placental site on the ratios. However the ratios of previous studies do not seem to be practical ones as CRL/BPD, AC/BPD, and CRL/AC. On the other hand, good reproducibility of most measurements of fetal biometry in early pregnancy by abdominal ultrasound has been demonstrated [4, 8]. CRL and BPD showed high reproducibility beginning at nine weeks of gestation onwards, whereas AC is only reliable from 11 weeks onwards, but, FL showed poor reproducibility before 14 weeks of gestational age. Therefore the ratios CRL/BPD, AC/BPD, and CRL/AC seem to be reliable as well.

Table 3. — Comparison of biometric ratios among some studies.

<table>
<thead>
<tr>
<th>Week</th>
<th>HC/AC</th>
<th>BPD/FL</th>
<th>FL/CRL</th>
<th>FL/AC</th>
<th>HC/FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>11º-11º</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>5th percentile</td>
<td>1.1 * 1.1</td>
<td>2.6 * 2.5</td>
<td>3.4</td>
<td>7.2 * 7.6</td>
<td>8.1 * 7.8</td>
</tr>
<tr>
<td>Median</td>
<td>1.2 * 1.3</td>
<td>3.7 * 3.3</td>
<td>4.8</td>
<td>9.6 * 9.7</td>
<td>10.8 * 10.0</td>
</tr>
<tr>
<td>95th percentile</td>
<td>1.4 * 1.4</td>
<td>5.2 * 4.3</td>
<td>7.7</td>
<td>14.5 * 12.6</td>
<td>16.1 * 13.0</td>
</tr>
<tr>
<td>12º-12º</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>5th percentile</td>
<td>1.1 * 1.1</td>
<td>2.3 * 2.2</td>
<td>2.6</td>
<td>6.7 * 6.6</td>
<td>6.9 * 6.7</td>
</tr>
<tr>
<td>Median</td>
<td>1.2 * 1.3</td>
<td>3.2 * 2.9</td>
<td>3.3</td>
<td>8.7 * 8.6</td>
<td>8.8 * 8.8</td>
</tr>
<tr>
<td>95th percentile</td>
<td>1.4 * 1.4</td>
<td>4.2 * 3.7</td>
<td>4.8</td>
<td>12.3 * 11.2</td>
<td>12.0 * 11.2</td>
</tr>
<tr>
<td>13º-13º</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>5th percentile</td>
<td>1.1 * 1.1</td>
<td>2.1 * 1.9</td>
<td>2.2</td>
<td>6.0 * 6.0</td>
<td>6.1 * 5.9</td>
</tr>
<tr>
<td>Median</td>
<td>1.2 * 1.3</td>
<td>2.7 * 2.5</td>
<td>2.7</td>
<td>7.6 * 7.4</td>
<td>7.5 * 7.4</td>
</tr>
<tr>
<td>95th percentile</td>
<td>1.4 * 1.4</td>
<td>3.4 * 3.2</td>
<td>3.4</td>
<td>10.2 * 10.2</td>
<td>9.6 * 10.2</td>
</tr>
<tr>
<td>14º-14º</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>5th percentile</td>
<td>1.1 1.12</td>
<td>1.9 1.7 2.0</td>
<td>5.3 * 5.4 4.82</td>
<td>* * 6.08 6.9</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.2 1.23</td>
<td>2.3 1.87 2.3</td>
<td>6.5 * 6.5 5.4</td>
<td>* * 6.55 8.1</td>
<td></td>
</tr>
<tr>
<td>95th percentile</td>
<td>1.4 1.33</td>
<td>2.8 2.06 2.8</td>
<td>8.3 * 8.0 6.04</td>
<td>* * 7.05 9.9</td>
<td></td>
</tr>
</tbody>
</table>

a: von Kaisenberg et al.; b: Snijders et al.; c: Present Study; d: Johnsen et al.; ¶: The ratios were recalculated as 1/ratio in study.

References


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Conclusion

The ratios of CRL/BPD; AC/BPD, and CRL/AC seem more practical to be used in the first trimester fetal ultrasonography practice to check for abnormal or mistaken measurements.
Immunohistochemical study of Inhibin A and B expression in placentas from normal and pathological gestations

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C. Kleanthis¹, D. Hassiakos²

¹Pathology Laboratory, Aretaieion Hospital, University of Athens, Medical School, Athens
²Second Department of Obstetrics-Gynaecology, Aretaieion Hospital, University of Athens, Medical School, Athens (Greece)

Summary

Objective: The aim of the study was to examine, by an immunohistochemical method, the distribution of Inhibin-A and -B, in placentas from normal and pathological gestations. Materials and Methods: Sixty-two specimens of placental tissue were examined: i) ten cases from early gestations, ii) 28 cases from mature placentas, iii) six cases associated with intrauterine growth restriction, iv) four cases associated with diabetes mellitus and v) 14 placentas from gestations with fetal chromosome abnormalities. The expression of Inhibin A and B was studied by automatic Ventana method. Results: i) Early gestation specimens: Inhibin A (+) immunoreaction was observed in the syncytiotrophoblast (8/10 cases) and in the intermediate trophoblast (6/10 cases). Inhibin B (+) immunoreaction was observed in the syncytiotrophoblast (10/10 cases) and in the intermediate trophoblast (4/10 cases). ii) Normal mature placentas: Inhibin A (+) immunostain was observed in 2/28 cases in the syncytiotrophoblast and in 7/28 cases in the intermediate trophoblast. Inhibin B (+) immunostain was observed in 28/28 cases in the syncytiotrophoblast and in 18/28 cases in the intermediate trophoblast. iii) Placentas associated with intrauterine growth restriction: Inhibin A (+) immunostain was observed in the intermediate trophoblast in 6/6 cases and in 4/6 cases in the intermediate trophoblast. iv) Placentas associated with gestational diabetes mellitus: Inhibin A (+) immunostain was observed in 2/4 cases in the syncytiotrophoblast and in 4/6 cases in the intermediate trophoblast. v) Placentas from pregnancies associated with fetal chromosome abnormalities: no Inhibin A immunoreaction was observed. Inhibin B (+) immunostain was observed in 13/14 cases in the syncytiotrophoblast and in 9/14 cases in the intermediate trophoblast. Discussion: Inhibin A and B are located in the syncytiotrophoblast and the intermediate trophoblast of the placenta, during early pregnancy (Inhibin A) and present throughout pregnancy (Inhibin B). No remarkable findings in placentas of pathological gestations support the evidence that Inhibins do not participate in processes that affect the development of the placenta or the feto, but may participate in the mechanism of labor.

Key words: Inhibins; Placenta; Syncytiotrophoblast; Trophoblast; Diabetes mellitus; Intrauterine growth restriction.

Introduction

Inhibins are heterodimeric glycoprotein hormones of the transforming growth factor-b super family and consist of one a-subunit and two b-subunits with main action in the suppression of follicle-stimulating hormone (FSH) secretion [1, 2].

The placenta is the main source of Inhibins in the maternal circulation and all Inhibins isoforms are present in extracts of term human placenta and towards the end of human pregnancy, increasing concentrations of immunoreactive and bioactive Inhibins are present in maternal serum [1-4]. The exact source of the specific inhibin isoforms from the placental components is yet unclear. Several studies report that Inhibins are involved in the paracrine regulation of prostaglandin, human chorionic gonadotropin (hCG), progesterone (P) release, and the maintenance of pregnancy and subsequent initiation of labor [1, 5-8].

In this study the distribution of Inhibin-A and Inhibin-B was investigated in normal placentas by an immunohistochemical method, as well as in placentas from cases diagnosed with intrauterine growth restriction (IUGR), or gestational diabetes mellitus (GDM), or fetal chromosome abnormalities.

Materials and Methods

The authors examined Hematoxylin and Eosin-stained (H&E) sections from formalin-fixed and paraffin-embedded tissues from 62 placentas: i) ten from first trimester gestations, ii) 28 from normal full-term gestations, iii) six from pregnancies associated with IUGR, iv) four from pregnancies associated with GDM, and v) 14 associated with fetal chromosome abnormalities.

All cases were retrieved from archival material of the Aretaieion University Hospital Pathology Laboratory. Additional sections of each case were obtained for immunohistochemical investigation by Automated Ventana Immunostainer. Slides were incubated with primary mouse monoclonal anti-Inhibin β₁ subunit IgG2b antibody (Serotec, Oxford, England; batch 0694, clone E4, dilution 1:100) and monoclonal anti-Inhibin α subunit IgG2a antibody (Serotec, Oxford, England; batch 1097, clone R1, dilution 1:50). Positive controls (sections from normal human testis which showed typical strong immunostain of Leydig cells for both Inhibin-α and Inhibin-β subunit) and negative controls were also included with each staining procedure.

The percentage of Inhibin-immuno-positive cells was determined by two independent observers and subsequently graded as “focal” (< 25% of cells positive), “intermediate” (25% to 75% of cells positive), or “diffuse” (> 75% of cells positive) (Table 1).

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Results

Group A: Placentas from first trimester gestations - ten cases

A diffuse Inhibin-A positive immunostain was observed in 8/10 specimens located in the villous syncytiotrophoblast, and in 6/10 specimens located in placental intermediate trophoblast (Figure 1).

Inhibin-B positive immunostain of the syncytiotrophoblast was observed in all specimens (10/10) (Figure 2), and of intermediate trophoblast in 4/10 specimens. The cytotrophoblast and the various cellular components of the decidua, presented negative immunoreaction.

Group B: Placentas from normal term gestations - 28 cases

Inhibin-A positive immunostain was observed in 2/28 specimens located in syncytiotrophoblast and in 7/28 specimens located in intermediate trophoblast. The cytotrophoblast, cellular components of the decidua, umbilical cord, and membranes presented negative immunoreaction.

Inhibin-B positive immunostain was observed in the syncytiotrophoblast in all 28 specimens and in the intermediate trophoblast in 18/28 specimens. The cytotrophoblast, cellular components of the decidua, umbilical cord, and membranes presented a negative immunostain reaction.

Group C: Placentas from IUGR gestations - six cases

Only the intermediate trophoblast showed a positive Inhibin-A immunostain, observed in 2/6 specimens. The other villous and placenta components were negative.

Inhibin-B positive immunostain was observed in syncytiotrophoblast in 5/6 specimens and in intermediate trophoblast in 4/6 specimens. All other placental components presented a negative immunostain reaction.

Group D: Placentas from pregnancies complicated with GDM - four cases

Inhibin-A positive immunostain was observed only in intermediate trophoblast in 2/4 specimens. All other placental components presented a negative immunostain reaction.

Inhibin-B positive immunostain was observed only in the syncytiotrophoblast in 2/4 specimens.

Group E: Placentas associated with fetal chromosomal abnormalities - 14 cases

All placental components presented a negative Inhibin-A immunostain reaction.

Inhibin-B positive immunostain was observed in syncytiotrophoblast in 13/14 specimens and in the cytotrophoblast in 5/14 cases. A positive focal immunostain was observed in intermediate trophoblast of the decidua in 9/14 cases.

Discussion

Inhibins are glycoprotein heterodimeric hormones that belong to the super family of the β transforming growth factor (TGF-β). They were initially detected by their effect of down-regulation on FSH.

Inhibins present two subunits: a common subunit α and two subunits βA and βB. The combination of these subunits gives rise to two different dimmers, Inhibin-A (α-βA), and Inhibin-B (α-βB), respectively. The two subunits are peptidic chains connected by double sulphide attachments. The molecular weight of subunit α and β is 18 kDa and 14 kDa respectively, and when combined, a mature Inhibin molecule of 32 kDa is created.

The two subunits are produced by two different genes and when separate, they do not present any biological activity. The heterogeneity of Inhibin can also be attributed to the action of proteases, to which this molecule is subjected during its passage through the vascular tree.
Immunohistochemical study of Inhibin A and B expression in placentas from normal and pathological gestations

<table>
<thead>
<tr>
<th></th>
<th>Inhibin-A SC</th>
<th>Inhibin-B SC</th>
<th>Inhibin-A IMT</th>
<th>Inhibin-B IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>80%</td>
<td>60%</td>
<td>100%</td>
<td>40%</td>
</tr>
<tr>
<td>Group B</td>
<td>7%</td>
<td>25%</td>
<td>100%</td>
<td>64%</td>
</tr>
<tr>
<td>Group C</td>
<td>0%</td>
<td>33%</td>
<td>83%</td>
<td>67%</td>
</tr>
<tr>
<td>Group D</td>
<td>0%</td>
<td>50%</td>
<td>50%</td>
<td>0%</td>
</tr>
<tr>
<td>Group E</td>
<td>0%</td>
<td>0%</td>
<td>93%</td>
<td>64%</td>
</tr>
</tbody>
</table>

Group A: First trimester placentas, Group B: last trimester placentas, Group C: placentas from IUGR pregnancies, Group D: placentas from pregnancies with GDM and Group E: placentas from pregnancies with fetal chromosomal abnormalities.

There is evidence that only a part of circulating Inhibin is biologically active as a dimer [1-3]. Inhibin is produced by the fetomaternal unit during pregnancy. Its circulating levels reach their maximum during the first week of gestation. Beyond this gestational age they decline and remain stable until the 25th week from which they rise up again until the completion of gestation. Inhibin, detected in the maternal serum throughout this period, is produced exclusively by the placenta, and studies have shown that placental trophoblastic cells of term gestations do actually secrete Inhibin.

Inhibin acts by down-regulating the secretion of FSH and by up-regulating the secretion of gonadotropin-releasing hormone (GnRH) from the central nervous system, of P by the placenta, and of hCG. In vitro experiments show that Inhibin drastically down-regulates the production of hCG by the placenta at the end of gestation, a fact that is not supported by other studies. In cases of molar pregnancy, high levels of circulating Inhibins are detected, followed by high levels of hCG. Furthermore, there is evidence that Inhibin seems to regulate the release of prostaglandins as well.

It is generally accepted that Inhibins enroll a regulating action of paracrine and endocrine functions during pregnancy. In the amniotic fluid, both Inhibin-A and B are found in high concentrations. In the maternal serum on the other hand, only levels of circulating Inhibin-A are high. In the umbilical cord Inhibin-B is detected in male embryos only, while Inhibin-A cannot be traced in either male or female embryos. Additionally, Inhibin-A cannot be traced in embryonic circulation. Finally, both isoforms can be traced in placental villi.

Serum concentration of Inhibin-A during the eighth to ninth gestational weeks in normal pregnancies is 550 pg/ml, and it is considered a predictive index for a good gestational outcome after the 16th to 18th week. It must be noted that Inhibins are the products of the placenta and of the ovarian follicle and thus not traceable in the circulation of a male patient. Considerable reduction of the levels of Inhibin-A in the amniotic fluid is noted, when comparing gravidas during active labor with gravidas subjected to Caesarian section. This fact may be explained by an alteration of the relationship between Inhibin and activin, that is believed to be of crucial significance for the proper function of the fetomaternal unit, and for good gestational and obstetric outcomes [4-6].

Trophoblastic tissues are considered the main sites of production of Inhibins. Placental syncytiotrophoblast is the main site of production of Inhibin-A, while chorionic trophoblast and membranes produce both Inhibin-A and B, that are then released in the amniotic fluid. Placenta secreted Inhibin-A in high concentrations in the maternal circulation remains invariable during active labor.

In the embryonic circulation, secretion of Inhibin-B is sex-dependent and its main sources are the testicles and probably the lungs. No Inhibin-A can be traced in the embryonic circulation.

The results of this investigation show that Inhibin-A is mainly located in the syncytiotrophoblast, and in certain cases, in the placental intermediate trophoblast during the initial stages of gestation (first trimester). There is a gradual decrease of the immunoreaction until the middle of the gestation, from which point and until the end of pregnancy, it is no longer traceable.

On the other hand, Inhibin-B is detectable throughout gestation and presents a characteristic distribution. It is detectable mainly in the syncytiotrophoblast and less in the intermediate trophoblast, in the chorion laeve, and decidua. The cytotrophoblast, umbilical cord, and membranes present a negative immunostain in all cases.

From the examination of the specimens of the other Groups (IUGR, GDM, and fetal chromosomal abnormalities), no significant findings were observed, and this may insinuate that Inhibins do not participate significantly in the normal growth of the embryo and placenta, while they play a major role in sustaining a normal gestation and initiating the phenomenon of labor [9, 10].

Conclusion

In normal pregnancies, Inhibin-A is present in the syncytiotrophoblast and in the intermediate trophoblast of the placenta during the first gestational weeks (first trimester), and it gradually decreases until the first half of pregnancy where it is no longer detectable.

In contrast, Inhibin-B can be traced by immunohistochemistry during gestation, presenting a characteristic distribution, and being located in the syncytiotrophoblast and in smaller amounts in the intermediate trophoblast of the chorion laeve and the decidua.

No significant changes of these patterns in placentas of pathological pregnancies were observed and this supports the theory that Inhibins do not participate in the mechanisms needed for the proper growth of the embryo or the placenta, having an action limited probably to the initiation of active labor.

The other components of the placenta, the cytotrophoblast, umbilical cord, and membranes, do not participate in the production of Inhibin-A or B, as observed by the immunohistochemical investigation.
References


Ultrasound parameters and L/S ratio in prediction of perinatal outcome in term-growth restricted newborns

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3School of Medicine, University of Belgrade, Belgrade (Serbia)

Summary

Aim: The relation between biophysical profile (BPP), cerebroplacental (C/P) ratio, and lecithin/sphingomyelin (L/S) ratio as a predictor perinatal outcome in term intrauterine growth restricted (IUGR) neonates was evaluated. Materials and Methods: A retrospective study of the perinatal outcome of 77 term monofetal pregnancies complicated with IUGR fetuses (< 10 percentile) who were terminated by cesarean section in 2010 was performed at the Institute of Gynecology and Obstetrics, Belgrade. Results: The most frequent early neonatal complication was asphyxia. The authors found a strong correlation between the L/S ratio and birth weight (BW) r = 0.609, as well as between BPP and Apgar score 5 r = 0.583. Significant negative correlation was found between asphyxia and BPP r = -0.398, as well as between asphyxia and C/P ratio r = -0.379. Conclusion: In serious IUGR neonates, low values of BPP and L/S ratios predicted asphyxia.

Key words: Growth restricted neonates; Biophysical profile; Cerebroplacental ratio; Lecithin/Sphingomyelin ratio; Asphyxia; Respiratory distress syndrome.

Introduction

Intrauterine growth restriction (IUGR) is a serous complication of pregnancies which is associated with an increase in fetal and neonatal morbidities and mortality rates [1]. IUGR fetuses are either low-growth potential, as result of genetic disease or environmental damage, or due to reduced placental perfusion and utero-placental insufficiency, and are at increased risk of perinatal morbidity and mortality and will require close feto-maternal monitoring and probably earlier intervention. IUGR occurs when gas exchange and nutrient delivery to the fetus are not sufficient to allow it to thrive in utero. Intrauterine fetal demise, asphyxia, meconium aspiration, neonatal hypoglycemia, and hypothermia are increased in both preterm and term IUGR neonates [1, 2]. Ultrasonographic evaluation of the fetus is based on multi-etiology of this disorder and includes fetal biometry and amniotic fluid index if less than eight cm, can present early sign of decrease in fetoplacental circulation. In pregnancies complicated by placental dysfunction, there may be a reduction in the number of functional villi and/or small blood vessels with resulting increased impedance, mainly reflected by a decrease in end-diastolic velocity. When the resistance further increases, there is no diastolic forward velocity (absent end-diastolic velocity - AEDV). Further increase in the resistance causes reversed end-diastolic velocity (REDV) and middle cerebral centralization, a late step in the cascade of events leading to poor perinatal outcome [3]. Changes or absence of diastolic flow in the umbilical artery can be observed well before the biophysical profile (BPP) demonstrates abnormalities.

The cerebroplacental ratio (C/P) may change subtly in response to shifts in circulation, representing the earliest stages of brain sparing.

Changes in C/P ratio may provide an early warning system to initiate more detailed fetal surveillance using BPP score [4, 5]. The relative proportion of lecithin (disaturated phosphatidylcholine) and sphingomyelin are stable until the middle of the third trimester, at which time the pulmonary active phospholipid lecithin, increases relative to the non-pulmonary sphingomyelin. A lecithin/sphingomyelin (L/S) ratio of at least 2:1 is considered indicative of fetal maturity. L/S ratio is very important in diagnosis of fetal lung maturity and is indirectly determined by influence of fetal hypoxia [6].

The aim of the study was to evaluate the predictive value of ultrasonographic parameters resistance indices in umbilical and middle cerebral arteries, C/P ratio, BPP, and L/S ratio on early neonatal morbidity in term-growth restricted newborns.

Materials and Methods

Retrospective study of 77 term (36 to 42 weeks) single-live born IUGR neonates who were delivered by elective or urgent cesarean section in 2010 in this Institute. IUGR neonates were defined as birth weight (BW) below the 10th percentile for gestational age. The authors analyzed: maternal age, parity, maternal morbidity, gestational age at delivery, BW, Apgar score at 5th minute, and early neonatal morbidity. Antepartum fetal assessment was performed using: fetal biometry, amniotic fluid index, BPP, resistance indices in umbilical and middle cerebral arteries, the C/P and L/S ratios. Fetal weight was estimated using Hadlock’s method based on measurements of the fetal head, body, and femur. The fetal BPP was performed daily for 20 min. A combined real-time pulsed Doppler system fitted with 3.75 MHz curvilinear probe was used. The spatial peak...
temporal average power did not exceed 87 mW/cm. The Doppler angle of insonation was less than 30, the sweep speed was 2.5 cm/s, and the pulse repetition frequency ranged from 3.5 KHz to 5.0 KHz. The women rested in semi-recumbent position during Doppler examination. The same physician performed all measurements during fetal apnea. Blood velocity waveforms were obtained from both umbilical and fetal middle cerebral arteries. The umbilical artery was insonated close to its placental insertions and the middle cerebral artery about one cm distal to its origin from the internal carotid artery. The resistance index was calculated for each vessel by averaging the first two good quality resistance indices obtained from two consecutive waveforms. The C/P ratio less than one was considered abnormal. The last Doppler examination and the newborn evaluation were performed within five days of delivery. The specimens of amniotic fluid for estimate L/S was obtained by amniocentesis, 24 hrs before delivery. L/S ratio was estimated by Clemet’s shake test.

Statistical analyses were performed using: Spearman’s Rho-, and Student’s t-test. Continuous variables were presented as mean (standard deviation as 95% confidence interval) assessed for normality or not. The Spearman’s test of correlation was used to estimate significance of correlation between neonatal BW and L/S ratio (p = 0.000) (Figure 2). C/R ratio less than one was diagnosed in 24.7% (19/77) fetuses. Apgar score at 5th min less than 7 occurred in 11 (14.3%) neonates. BPP showed significant correlation to low Apgar score 5 (p < 0.05). A significant negative correlation was found between frequency of asphyxia and BPP (p = 0.000), C/P ratio (p < 0.05), and L/S ratio (p < 0.05). Increasing incidence of RDS was in a statistically-negative correlation with L/S ratio (p < 0.05), BPP (p = 0.003), as well as with C/P ratio (r < 0.05). Elective cesarean sections were performed in 47/77 (61%) patients.

Discussion

The incidence of IUGR neonates increased more in nulliparous patients than in multiparous patients older than 30 years [7]. The growth restricted fetus can experience numerous complications in the neonatal period related to antepartal and intrapartal factors [4, 5]. Attempting to identify the cause of IUGR is important because it may have an influence on the estimate of recurrence and future pre-conceptional counseling, pregnancy management, prenatal diagnostic procedures, and neonatal management [7]. The majority of women (90.1%) had adequate prenatal care in pregnancy.

BPP scores were low in most of the high-risk pregnancies. Decrease in amniotic fluid index is long-term effect of chronic fetal hypoxia. Changes in the fetal heart rate are the first reactions to hypoxia regardless of the etiology [8]. BPP score less than four was diagnosed in 19.5% of IUGR fetuses in this study. Declining BPP scores strongly predicts increasing frequency of fetal distress and low five-minute Apgar score [5]. The authors found strong correlation between BPP and Apgar score 5 which was important for estimation of early and late complications among IUGR neonates.
The most frequent early neonatal complication was perinatal asphyxia in 57% but C/P ratio less than one was diagnosed in 24.7% fetuses. The combination of small abdominal circumference, normal anatomy, low BPP score values, and abnormal umbilical artery Doppler recording is strongly-suggestive of fetal IUGR due to placental insufficiency, i.e., in 24.7% of cases. Doppler may distinguish between small normal fetuses that will not manifest any abnormal placental vasculature, and fetuses affected by conditions causing restricted growth, secondary to placental condition and who are thus at higher risk of intrapartal difficulties and perinatal mortality [9]. The C/P ratio less than one provides identification of IUGR fetuses who are at higher risk for perinatal asphyxia. Changes in C/P ratio indicates the need for more intensive fetal monitoring using BPP which was also shown in this study [5]. Low BPP scores can also predict development of RDS as a result of prolonged intrauterine hypoxia.

Prolonged intrauterine hypoxia is inhibitor of synthesis components of surfactant and causes L/S ratio less than two, considered fetal immaturity and high-risk for neonatal asphyxia as in this study [6].

Conclusion

The risk of perinatal asphyxia and RDS were higher in IUGR neonates with lower BPP. Umbilical artery Doppler is a relative predictor of IUGR due to placental dysfunction, as well as the C/P ratio as relative predictor of neonatal asphyxia.

L/S ratio less than two as a result of chronic intrauterine hypoxia is associated with increased incidence of asphyxia and RDS in term IUGR neonates. It seems that neonatal BW is the best predictor of pulmonary maturity in high-risk pregnancies.

References


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Symptomatic Shigella sonnei urinary tract infection in pregnancy

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Introduction
Shigella bacteria rarely cause urinary tract infection (UTI) since they usually are responsible for gastrointestinal infections [1, 2]. In particular, UTI caused by Shigella sonnei is uncommon. We report a case of UTI due to Shigella sonnei in a pregnant woman, a rather unusual finding.

Materials and Methods
A 31-year-old female (gravida 3, para 2) presented to our hospital complaining of left-flank tenderness, dysuria, and fever (temperatures at home peaked at 39.0°C). She had no diarrhea or vomiting. Since she was in the second trimester of pregnancy (24 weeks of gestation), she was admitted for further investigation.

Results
On admission, the physical examination revealed a temperature of 38.2°C, a pulse of 64/min, a blood pressure of 110/60 mm Hg, 20 respirations per minute, and left-flank tenderness while significant laboratory data was collected: leukocyte count 10,800/ul with 86% neutrophils, erythrocyte sedimentation rate 59 mm/h, serum creatinine 0.6 mg/dl, albumin 4.0 g/dl, and C-reactive protein 9.6 mg/dl. Urinalysis revealed 30 to 50 leukocytes per high power field while from the quantitative urine culture Shigella sonnei was recovered after 24 h incubation at 37°C. After a two-week course with 750 mg cefuroxime every 8 h, the patient experienced gradual resolution of all symptoms and urinary cultures were negative two weeks and one month, respectively, after completing the therapy. The gestational course was uneventful and the patient delivered a healthy baby girl at term.

Discussion
Shigella species represent highly communicable pathogens that usually cause gastrointestinal infections and are rarely responsible for UTI [1, 2]. In particular, Shigella sonnei UTI is uncommon [1, 3, 4] and, at present, there are only very few cases of UTI due to Shigella sonnei reported in the literature so far. Including the present case, we are aware of only ten reported cases.
Symptomatic Shigella sonnei urinary tract infection in pregnancy

117

[1, 3-8]. Of these, five occurred in adults, one male, and four females. Our case is the first to present an UTI due to Shigella sonnei during pregnancy. The remaining five cases included children, all females, aged between two months and six years.

We report the case of a 31-year-old female in the second trimester of pregnancy with symptoms of acute pyelonephritis. It has been demonstrated that infections with Shigella species during pregnancy can result in complications such as preterm premature rupture of membranes and preterm delivery [9]. In our patient, Shigella sonnei was isolated only from the urine culture. However, this case is interesting since the patient was not a fecal carrier and had no history of dysentery. Thus, the time and source of Shigella sonnei as well as the mechanism of infection in our patient remain unknown.

The mode, in which Shigella species gain access to the urinary tract and UTI occurs, is still unclear. Especially in pregnant women, it seems that the ascending retrograde route might be the most probable way of infection [1]. This could be a plausible scenario since we encounter reported cases of Shigella vaginitis in the literature [10]. Other possible mechanisms by which these microorganisms might gain access to the urinary tract are bacteremia and sexual transmission [11-13]. Shigellemia is rare and neither of the aforementioned possibilities could be demonstrated since our patient had no signs of bacteremia and the pathogen could not be isolated from blood cultures or from any member of the family, in particular from her husband.

Conclusion

Shigella sonnei can cause UTI during pregnancy even in the absence of predisposing factors or an apparent source of infection. The response to therapy suggests that eradication of this pathogen from the urinary tract is easy.

References


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Single dose epidural morphine instead of patient-controlled epidural analgesia in the second day of cesarean section; an easy method for the pain relief of a new mother

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Summary

Purpose: Pain management has a particular importance after Cesarean section. This study was undertaken in order to document the efficacy and side-effects of epidural morphine instead of patient-controlled analgesia technique used for the control of post-cesarean pain during postoperative 24-48 hours. Materials and Methods: This study was performed as a retrospective review of patient charts who had received combined spinal-epidural anaesthesia. Post-cesarean analgesia was performed with epidural technique either by using (Group 1) patient-controlled epidural analgesia for 48 hours, or (Group 2) patient-controlled epidural analgesia for the first 24 hours and then single dose of 3 mg epidural morphine for the second 24 hours. Results: Incidences of side-effects were similar in both groups. None of the patients experienced respiratory depression. Additional analgesia was used on an as-required basis in nine of 39 (23%) patients in Group 1 and six of 39 (13%) in Group 2. Conclusion: Small doses of epidural morphine provides up to 24 hours of pain relief from a single injection and could obviate the need for an indwelling epidural catheter on the second day of post-cesarean section, thus reducing the potential for catheter-related complications.

Key words: Postoperative analgesia; Cesarean section; Epidural; Morphine.

Introduction

Cesarean section usually causes moderate to severe postoperative pain continuing for up to 48-72 hours [1, 2]. The new mother has responsibility of caring for her newborn baby. Pain makes it difficult for the new mother to optimally care during the postoperative period and may adversely affect early interaction between mother and infant [2]. Good pain control hastens ambulation, decreases maternal morbidity, improves patient outcome, and facilitates care of the newborn.

There are a variety of techniques and agents used for post-cesarean pain management. Because pain is often more intense during the first two days after cesarean section, the use of an indwelling epidural catheter is usually a requirement for postoperative pain management. Among patients who receive regional anaesthesia, the indwelling epidural catheter facilitates the intermittent bolus injection or continuous epidural infusion of opioids or local anaesthetics during the postoperative period. However, the epidural catheter and patient-controlled epidural analgesia (PCEA) have been associated with several problems like catheter migration, accidental dislodgement, infection, and limited mobilization of the new mother. A local anaesthetic with opioid provides effective analgesia but concentration of local anaesthetics may lead to sensory and motor blockade. Although it is not a bothersome issue in the early hours of postoperative period, mobilization and ambulation of the new mother will soon be important for the care of the newborn.

Concerns about undesirable consequences associated with prolonged PCEA may force the practitioners to remove the epidural catheter earlier in the postoperative period, but realize that the new mother will still have intense pain that can limit the mobilization and the ability to optimally care for her infant on the second day. For these patients, an opportunity exists to administer a long-acting opioid in the epidural space before removal of the catheter.

The author considered that as compared to the combination of local anaesthetic and opioid by the patient-controlled epidural analgesia technique, single dose epidural morphine for postoperative analgesia should provide better pain control and satisfaction (owing to increased mobility), and less risk of motor and sensory blockade. This retrospective report was undertaken in order to document the efficacy and side-effects of epidural morphine instead of PCEA technique when used for the control of post-cesarean pain during postoperative 24-48 hours.

Materials and Methods

This study was performed as a retrospective review of patient charts who had received combined spinal-epidural anaesthesia and postoperative epidural analgesia for cesarean section at the present University hospital. The charts of 78 patients were reviewed.

A standardized data collection form was completed for each patient and the variables included: patient demographics, heart rate, blood pressure, respiratory rate, pruritus, urinary retention, nausea and vomiting, time and dose of supplemental analgesics administered during the 24-48 hours, and time of accidental epidural catheter dislodgement were recorded.

Neuraxial techniques were performed by anaesthesiologists.
and residents supervised by a staff anaesthesiologist. For combined spinal-epidural analgesia, after location of the epidural space at the L3-L4 or L4-L5 interspace, dural puncture was performed. Then a spinal injection of bupivacaine 8 mg with fentanyl 20 μg was administered intrathecally and the spinal needle was removed. An epidural catheter was introduced 2-3 cm into the epidural space. Surgery was allowed to begin after the patient developed satisfactory block at least at the T5-6 level.

Post-caesarean analgesia was performed with an epidural technique either by using (Group 1) patient-controlled epidural analgesia for 48 hours, or (Group 2) patient-controlled epidural analgesia for the first 24 hours, and then single dose epidural morphine 3 mg for the second 24 hours. In both of the groups, the epidural catheter was connected to a patient-controlled analgesia (PCA) pump, set to deliver a continuous infusion of 7 ml/hr of 0.05% bupivacaine and fentanyl 1.5 μg/ml. The patient-controlled bolus was 7 ml and the lock-out time was 20 minutes. The patient-controlled epidural analgesia was continued in Group 1 for 48 hours and in Group 2 for 24 hours. Morphine was administered through the indwelling epidural catheter as a single 3 mg dose in a 5 ml volume at the 24th hour after surgery in Group 2 and then the epidural catheter was removed.

After discharge from the recovery room, vital signs were monitored by postpartum unit nurses. Also, all patients were interviewed by a nurse or by an anaesthesiology resident for 48 hours. They were asked about the side-effects and their satisfaction with pain relief.

Before placement of the epidural catheter, baseline pain was assessed by Verbal Rating Scale (VRS; 0 = no pain, 10 = worst pain imaginable). Effective pain relief was considered when the patient had VRS < 3. If the patient had inadequate analgesia, supplementary rescue analgesia with oral paracetamol was available. Patient supplemental analgesic requirement times were recorded. Severe vomiting defined as more than two episodes in 24 hours was treated with intravenous metoclopramide 10 mg.

Patients were monitored for respiratory depression using pulse oximetry and respiratory rate. Respiratory depression was defined as either a SpO2 < 85% or respiratory rate < 10 breaths/min. Intravenous naloxone was readily available to be administered in the event that patients suffered from respiratory depression.

All statistical analyses were performed using SPSS for windows 13.0. Data were analyzed using independent Student’s t-test and Chi-square test. A p value of < 0.05 was considered statistically significant. The results are expressed as means with (±) standard errors.

Results

Seventy-eight patients undergoing cesarean section were included in this retrospective observational study (39 in each group). There were no significant differences between groups in maternal demographic characteristics (Table 1). No difference in the quality of sensory and motor block before and during surgery was noted in all patient records. No complications from the combined spinal-epidural block were noted. Systolic, diastolic blood pressures, heart rates, and oxygen saturations remained stable and there was no significant perioperative difference between the two groups (p > 0.05). There were no cases of significant hemodynamic instability during the postoperative 48 hours and arterial blood pressures were similar during this period.

The incidence of nausea, vomiting, pruritus, and urinary retention related to the use of epidural opioids are presented in Table 2. Nausea affected eight percent of patients in Group 1 and five percent in Group 2 (p > 0.05). Incidence of vomiting was similar in both groups (p > 0.05). Three patients in Group 1 and five patients in Group 2 complained of pruritus. Also, the incidence of urinary retention was similar in both groups (p > 0.05). None of the patients experienced respiratory depression.

Additional analgesia in the form of oral paracetamol was used on an as-required basis in nine of 39 (23%) patients in Group 1 and six of 39 (13%) in Group 2 on the second day after cesarean section.

Discussion

This retrospective study has shown that 3 mg of epidural morphine provides up to 24 hours of pain relief from a single injection and could obviate the need for an indwelling epidural catheter on the second day of postcesarean section, thus reducing the potential for catheter-related complications.

Besides other common disadvantages of postoperative pain as atelectasis, pneumonia, and thromboembolic complications, pain management has a particular importance after cesarean section. In obstetric patients, persistent pain interferes with early postpartum interaction between mother and baby [3] and it has shown that infant care and breast-feeding success can be improved by superior postoperative analgesia [4]. On the contrary, unsatisfactory pain control may lead to adverse cognitive changes, such as increased new mother’s anxiety. Anxiety and discomfort caused by pain may reduce the mother’s ability of effective breast-feeding. These activities are also hampered by the excess sedation and other side-effects associated with the administration of analgesic agents used in different routes. Nursing mothers are especially concerned about neonatal exposure to analgesic drugs, and this fear impels most of them to avoid medications that may accumulate in breast milk [3].

All of these factors render it difficult to designate an

| Table 1. — Demographic data values are expressed as mean ± SD. |
|-------------------------|-------------------------|
|                         | Group I (n = 39)         | Group II (n = 39)     |
| Age (years)             | 30.3 ± 6.0              | 29.5 ± 5.3            |
| Height (cm)             | 162.5 ± 5.7             | 160.1 ± 5.8           |
| Weight (kg)             | 75.8 ± 7.9              | 73.4 ± 8.4            |

*p < 0.05.

| Table 2. — Side-effects of epidural opioids. |
|-------------------------|-------------------------|
|                         | Group I (n = 39)         | Group II (n = 39)     |
| Nausea, n (%)           | 3 (8%)                  | 2 (5%)                |
| Vomiting, n (%)         | 1 (3%)                  | 1 (3%)                |
| Pruritus, n (%)         | 3 (8%)                  | 5 (13%)               |
| Urinary retention, n (%)| 1 (3%)                  | 1 (3%)                |

*p < 0.05.
optimal analgesic technique and agent for post-cesarean pain relief. In general, the method of postoperative pain control is determined by different factors such as: patient characterization, availability of the drug or resources, institutional protocols, individual preferences, and financial considerations. Clearly, PCEA is one of the most effective techniques for post-cesarean analgesia in many clinics. Whether it offers distinct advantages over other analgesic techniques, it also has some disadvantages like the necessity to maintain a functioning epidural catheter during the postoperative period [5]. As Miaskowski et al. reported that dislodgement and migration of epidural catheters have resulted in a high catheter failure rate reaching 25%, adding to the nursing time required for already-intensive analgesic modality [6]. Many studies describe the late migration of a well-functioning epidural catheter into the intravascular space [7, 8]. On the other hand, PCEA may run for many hours, at constant infusion rates, and with on-demand bolus doses administered by the patient. This may lead to an unnecessary accumulation of the drug as a result, unless the anaesthesiologist is able to periodically assess and manually readjust infusion and bolus settings on the epidural pump devices [9]. Unfortunately, it is not practical in most circumstances and this may be a primary reason for the need of single dose injection. Also, the use of single dose epidural technique reduces the potential resource utilization that is encountered with PCEA technique.

Epidural administration of local anaesthetics, in most cases with opioids, is a commonly-used modality for post-cesarean section analgesia. The concentration of local anaesthetics added to PCEA must be low to avoid significant sensory and motor blockade, because many patients are ambulatory within hours of surgery. On the other hand, with a lower concentration of local anaesthetics, ambulation may not be affected, but in this situation opioid concentration had to be increased for pain relief of the new mother. This may give rise to another problem associated with the high-dose neuroaxial opioid related side-effects like respiratory depression, pruritus, nausea, vomiting, sedation, and urinary retention [10-12].

Morphine is the most widely used epidural opioid for post-cesarean analgesia and pain relief from this agent follows a consistent dose-response relationship [5]. It is more advantageous compared to local anaesthetics as it has the advantages of producing analgesia without motor and sensory blockade or interference with neuromuscular function or causes depression of sympathetic nervous system [13]. Administering a long-acting opioid, such as morphine, into the epidural space and securing an efficient analgesia before removal of the catheter is an attractive opportunity, but an optimal effective dose of epidural morphine is still a matter of controversy with regards to its duration of action and side-effects. Studies have found that epidural morphine had an effective analgesic technique after cesarean section, with 3 mg being the optimal dose [1, 14]. However single dose neuroaxial techniques have an inherent disadvantage of providing limited period of analgesia. It is demonstrated that, with the usual dose range, epidural morphine is insufficient to achieve more than 30 hours pain-free. It is consequently not sufficient on the second postoperative day, when patients begin to care for their newborns [1, 15]. Administering of a higher-dose of morphine may provide more satisfactory, longer lasting analgesia to all patients, but at the cost of a higher incidence of undesirable side-effects.

As it is reported that cesarean section induces moderate to severe postoperative pain for 48 hours [1], its control is an indispensable necessity for the new mother. Therefore, practitioners need something that prolongs an analgesic effect for at least for 48 hours, preferably without depending on a device or a catheter. In this manner, using a continuous epidural technique during the first post-cesarean day and preferring a single dose long acting epidural technique for later period becomes more attractive. Additionally, patients may be able to transition directly to oral medications, because of the prolonged analgesic activity of morphine with a single dose. The simplified technical aspects of this therapy, when compared with prolonged continuous epidural infusion, may result in fewer adverse events and complications related with indwelling epidural catheter.

The effectiveness of epidural morphine in the control of post-cesarean pain is documented by the request of additional analgesic in this study. The incidence of the additional analgesic seems to be different in PCEA and epidural morphine used in patients (23% vs 13%). Epidural single dose morphine was well-tolerated in all patients, and found no significant differences of adverse events between PCEA group. The most consistent disadvantage of using epidural opioid is its increased rate of pruritus and the incidences of this complication were not statistically different in this retrospective study (8% vs 13%). Nausea and vomiting are well-documented side-effects of epidural morphine [5, 13]. The rates of these complications were also similar in epidural morphine and PCEA used patients (8% vs 5% for nausea and 3% vs 3% for vomiting).

Respiratory depression is the most feared side-effect of epidural morphine. The potentially life-threatening side-effect of delayed respiratory depression did not appear with epidural morphine used in these patients. Nevertheless, Kumarasamy et al. [13] reported that respiratory depression does not have the same incidence nor severity as seen in other surgical patients, since obstetric patients are relatively younger, healthier, and are motivated to care for their neonates. Additionally, progesterone has shown to improve ventilation by increasing the sensitivity of the respiratory centre to CO₂ [13].

There are several limitations of the present study that must be considered. Firstly, this is a retrospective, observational, and non-randomized study and the choice of analgesic technique was left to the individual anaesthesiologist. Secondly, because of the retrospective nature of the study, the quality of pain relief could not be objectively assessed, making it impossible to know whether the patients in PCEA group received equivalent analgesics. The total amount of local anaesthetic and opioid
Single dose epidural morphine instead of patient-controlled epidural analgesia in the second day of cesarean section; etc.

administered via PCEA could not be calculated because of the variability of bolus demand doses used for each patient. The third limitation is that standard dose morphine was used in all patients. As Stamenkovic et al. criticised themselves in their report, a ‘one-size fits all’ approach to postoperative analgesia may result in pain under-treatment for some patients, because of significant analgesic requirement variability between patients [16]. In this manner, frequent patient visits and additional analgesics used on an as-required basis were the main rescuers for the determination of unsatisfactory pain relief in this study.

This retrospective study did not aim to discuss the efficacy of epidural morphine. Morphine has long been used for postoperative analgesia for post-cesarean analgesia. It has been demonstrated in many studies that the quality of analgesia from epidural morphine is no less than that from continuous epidural analgesia [5, 14]. The main principle was to comfort the patient by satisfactory pain relief, less risk of motor and sensory blockade, and consequently earlier mobilization of the new mother. Increasingly, the trend to reduce the length of hospital stay with enhanced recovery surgery is being introduced for cesarean section. The reduction in hospital stay includes factors, such as quality of analgesia and absence of epidural motor blockade allowing for early mobilization. On the other hand, epidural single dose morphine should be found more cost-effective than the PCEA set-up.

In conclusion, the use of single dose epidural morphine for postoperative analgesia should provide better pain control and satisfaction (owing to increased mobility), less risk of motor and sensory blockade. Small doses of epidural morphine provide up to 24 hours of pain relief from a single injection and could obviate the need for an indwelling epidural catheter on the second day of post-cesarean section, thus reducing the potential for catheter-related complications. Ideally randomized and prospective studies should be performed to determine the exact role of single dose epidural morphine compared with patient-controlled epidural analgesia technique for the control of post-cesarean pain during postoperative 24-48 hours.

References
The efficacy of intrauterine versus oral progestin for the treatment of endometrial hyperplasia. A prospective randomized comparative study

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Summary

Objectives: This study compared the efficacy of the levonorgestrel-releasing intrauterine device (LNG-IUD) to oral medroxyprogesterone acetate (MPA) applied for the same length of time for the management of endometrial hyperplasia without atypia. Study Design: This was a single-center, open, randomized, and clinical trial. One hundred four patients aged between 30-50 years and diagnosed with endometrial hyperplasia without atypia by endometrial biopsy, were randomized to receive LNG-IUD or MPA. Both groups were further divided into two groups as three-month and six-month treatment subgroups. The primary objective was to compare the complete regression rates of hyperplasia, and the secondary objective was to determine the minimum duration of time required for the achievement of regression. Results: At two-year follow-up, the success rates of LNG-IUD treatment and oral MPA for three months therapy were 84% and 50%, respectively. While the regression rate was 100% in the six-month LNG-IUD group, it was 64% in the oral MPA group. LNG-IUD appeared to have a significantly higher success rate (p = 0.0001). Conclusion: It is believed that by this study LNG-IUD applications may be a reliable preference for younger patients who wish to preserve their uterus and especially for non-atypical cases, and if the patient demands fertility, even a six-month application will provide effective treatment.

Key words: Endometrial hyperplasia; Non-atypical; Therapy; Levonorgestrel-releasing intrauterine system; Oral progestin.

Introduction

Endometrial hyperplasia is thought to occur as the result of endometrial suffering due to a relatively strong estrogenic influence in the absence of adequate progesterone secretion. Aside from causing abnormal uterine bleeding, endometrial hyperplasia is an important clinical entity, since it may accompany estrogen-secreting tumors, and occur prior to endometrial cancers [1]. It has an increased prevalence between 40-55 years of age. In developed countries, approximately 200,000 new cases are estimated to occur each year [2].

The histologic changes in hyperplasias may vary from a mildly excessive appearance of endometrial proliferation to a complex structure including severe changes that can be hardly distinguished from a carcinoma [3]. As reported by the study of Kurman et al., the rate of progression to cancer is 1% for simple hyperplasia, 3% for complex hyperplasia, 8% for simple atypical hyperplasia, and 29% for complex atypical hyperplasia [4]. The appropriate treatment is decided by considering various characteristics of the patients such as age, hyperplasia type, and wish for future childbearing [5]. Most commonly preferred treatment methods include the administration of progesterone at varying doses and via diverse routes, or surgical intervention. Several recent studies have shown that intrauterine devices (IUDs) with the ability of releasing levonorgestrel (LNG) and other similar gestagens can be used in the treatment of hyperplasia without inducing a systemic side-effect that may be observed in oral progesterone administration [6, 7]. In the present study, in order to evaluate the efficacy of levonorgestrel-releasing intrauterine device (LNG-IUD) in a comparative fashion, the authors preferred medroxyprogesterone acetate (MPA) as the oral progesterone.

The first objective of this study was to compare the long-term outcomes of endometrial hyperplasia cases treated either with LNG-IUD or oral progesterone for the same length of time. Since patients of hyperplasia without atypia may have a wish for childbearing, the authors aimed to compare the long-term outcomes of short-term treatments by a prospective study including similar groups receiving the related therapy for same length of time. Therefore, the second objective was to determine the minimum duration of successful treatment in 30-50 age group with a wish for childbearing. The increasing fertility age of women in developed countries has contributed to the importance of this problem. The treatment success was defined as regression of endometrial hyperplasia. To the authors’ knowledge, this study is the first investigation to evaluate and compare the long-term outcomes of oral progesterone and LNG-IUD therapies applied for the same length of time against hyperplasia without atypia in the published literature.

Materials and Methods

Trial design

The authors designed an open-label, prospective, and randomized single-center trial for comparison of LNG-IUD and oral progesterone efficacies in the treatment of hyperplasia without atypia. The patients who presented to the Outpatient’s Clinic between January 2, 2005 – December 31, 2009 due to abnormal uterine bleeding and who were diagnosed with endometrial hyperplasia, were included in the study. Since most of the hyperplasia with atypia cases were generally above 40...
years of age, had no childbearing, and with a tendency to prefer hysterectomy due to cancer risk, the authors constituted the study population from cases of hyperplasia without atypia. Prior to the study, approval from the local ethics committee and informed consents from each patient were obtained.

This manuscript is reported according to the revised recommendations of the Consolidated Standards of Reporting Trials statement for improving the quality of reports of parallel group randomized trials [8].

Participants

The authors aimed to study patients below 50 years of age who were diagnosed with endometrial hyperplasia without atypia. Since one of the study objectives was to determine the minimum treatment duration in patients that might have a wish to remain fertile, the patients aged above 50 years, were excluded from the study. Among the individuals who presented abnormal uterine bleeding, 84 patients aged between 30-50 years and diagnosed as endometrial hyperplasia without atypia by endometrial biopsy, were included in the study. The patients with atypia, as well as those with submucous myoma, ovarian tumor, and a uterine myomatosis greater than 12 cm, were excluded from the study.

Symptoms, histopathologic results, and socio-demographic data (age, body mass index (BMI), parity, presence of diabetes or hypertension) of all the patients were recorded.

Interventions

Endometrial biopsy with a suction catheter was performed by a single investigator (KD) in patients that presented with abnormal uterine hemorrhage, and curettage was always recommended when the biopsy specimens were insufficient for diagnosis. The acquired histologic materials were evaluated by the gynecologic pathology department. Endometrial diagnosis was achieved by an experienced gynecologic pathologist (AB) based on the criteria defined by Kurman et al. [3]. Hyperplasia was categorized in two groups: hyperplasia with and without atypia. Each of the 16 patients with atypical hyperplasia who were aged above 40 years and had no concern about fertility, preferred to undergo hysterectomy.

In this study, two different treatment models, LNG-IUD and oral MPA, were investigated. Both of these two groups were further divided into two groups as three- and six-month treatment subgroups. LNG-IUD was left in the uterine cavity for three-months in Group 1 (n = 26) and six months in Group 3 (n = 26). Oral MPA treatment was applied for three months in Group 2 (n = 26) and six months in Group 4 (n = 26), with a dose of ten mg/day given ten days per month. Control biopsy for histologic assessment was performed four times in Groups 1 and 2: right after the treatment and at six, 12, and 24 months; whereas it was performed three times in Groups 3 and 4: right after the treatment and at 12 and 24 months. The histopathologic results of the control biopsies were assessed in three groups: persisting hyperplasia, regression of hyperplasia (an endometrium under the influence of gestagen or proliferative endometrium), and regression of hyperplasia where the hyperplasia recurs shortly after achievement of regression. In cases where hyperplasia regression could not be achieved, the patients were recommended to undergo continuous LNG-IUD replacement or hysterectomy.

Sample size

A power analysis was performed to determine the number of subjects required for the study. The primary objective was to compare the regression rates of endometrial hyperplasia without atypia. The previous studies have shown that oral MPA success in three to six month treatment is 48%-60%, whereas the success of long-term LNG-IUD therapy is known to be 63%-100% [2, 9]. Based upon those data, when the power of a test and level is recognized as 90% and 0.05 by the help of two-sided Chi-squared ($\chi^2$) test, the required sample size can be found as 96 patients (24 patients in each group).

Randomization

Eight of 112 patients who fulfilled the inclusion criteria, rejected to participate in the study after being informed about its design, and they preferred hysterectomy. The remaining 104 patients that provided an informed consent, were randomized. Randomization was conducted by using a computer-generated table of random numbers with allocation concealment.

Statistical analysis

Demographic and baseline data such as age, parity, BMI, diabetes, and hypertension, were compared. The data with normal distribution were expressed as mean and standard deviation values, whereas interquartile range was used for skewed data. The groups were analyzed by intention to treat. Accordingly, Wilcoxon (rank sums), $\chi^2$, and Fisher’s Exact tests were applied. As the changes in continuous variables were evaluated by paired t-test, categorical variables were evaluated by McNemar test. Kaplan-Meier survival curves were performed to calculate time to treatment failure and log-rank test was used to compare time to treatment failure between the groups. All statistical analyses were performed using SPSS version 15.0 software.

Results

Recruitment

Among 112 patients fulfilling the study inclusion criteria who presented between January 2, 2005 – December 31, 2009, 104 endometrial hyperplasia patients that provided an informed consent (61 simple and 43 complex), were included in the study and randomized into LNG-IUD and MPA groups, while eight patients rejected to participate in the study. As 102 of 104 patients completed the two-year follow-up period, one patient from Group 1 and another from Group 4 could not be reached and were both excluded from the study. The follow-up completion rate was 96.1% in Groups 1 and 4, whereas it was 100% in Groups 2 and 3; there was no statistically significant difference between the groups according to completion rate ($\chi^2$ test, $p = 0.834$).

The efficacy of intrauterine versus oral progestin for the treatment of endometrial hyperplasia. A prospective randomized etc. 123
Baseline data

The mean age of the patients was 43.5 years. There was no difference between the groups in terms of age, weight, BMI, parity, and menopausal status. As 87 (83.7%) of the patients were premenopausal, 17 (16.3%) were postmenopausal, and none of them had received tamoxifen or hormone replacement therapy prior to the study. The postmenopausal patients were not excluded from the study, since they had a chance of pregnancy with help of donor oocyte programs. Baseline characteristics are shown in Table 1.

Outcomes

The outcomes obtained throughout a two-year follow-up period are shown in Figure 1. At two-years follow-up, the success rates for the three-month LNG-IUD treatment in Group 1 and three-month oral MPA therapy in Group 2 were 84% (21 regression in 25 patients) and 50% (13 regression in 26 patients), respectively. The LNG-IUD treatment showed a statistically significantly higher success rate (p = 0.001). While the regression rate was 100% (26/26) in the six-month LNG-IUD group, it was 64% (16/25) in the oral MPA group. LNG-IUD appears to have a significantly higher success rate (p = 0.0001).

Treatment failure

Treatment failure was observed in 28 of 102 patients followed-up for two years. The failures were significantly less in the LNG-IUD compared to the MPA groups, with four out of 51 (7.8%) in the LNG-IUD compared to 22 out of 51 (43.1%) in the MPA group, with a hazard ratio of 0.45 (95% CI, 0.21-0.93, log-rank test p = 0.002) (Figure 2). Among four cases of failure in Group 1, one patient preferred hysterectomy and three patients opted for the reapplication of LNG-IUD. Among 12 cases of failure in Group 2, six preferred LNG-IUD and the other six preferred hysterectomy. Among eight cases of failure in Group 4, three preferred LNG-IUD and five preferred hysterectomy. Histopathologic examinations of the preoperative biopsy and postoperative hysterectomy materials were consistent in all 12 patients who preferred to undergo hysterectomy.

Survival analyses based on Kaplan-Meier and Cox proportional hazards model revealed shorter time to regression in the LNG-IUD group. There was no statistically significant relation between the regression rates and baseline covariates of survival analyses.

Discussion

After the fact that unopposed estrogen causing hyperplasia of the endometrium was discovered, oral proges-
terone supplementation became the mainstay for treatment. Without clear-cut consensus on the length and how much progesterone would suffice, hysterectomy remained a more reliable and definitive mode of treatment. After the advent of gestagen-releasing IUDs, it was readily foreseen that local delivery of high concentrations of progesterone for a local disease, such as endometrial hyperplasia, would be more logical. The success of LNG-IUD treatment in endometrial hyperplasia cases with or without atypia has been shown by various studies. Wildemeersch and Scarselli obtained successful results in all types of hyperplasias by inducing levonorgestrel release into the uterine cavity at a dose of 20 μg/day [10-12]. Compared with the systemic delivery option, using an IUD device for administration of progesterone has been shown to help attain higher concentrations in the endometrial tissue [13]. Although there are three previous studies comparing the efficacies of oral progesterone and LNG-IUD in endometrial hyperplasia treatment, this study is the first prospective study comparing the long-term outcomes of different treatments applied for the same length of time [2, 9, 14]. Orbo et al. [14] conducted a multicenter study in which the patients were divided into three groups and 85 patients received oral MPA ten mg/day for ten days per month (continued for three to six months), 66 received LNG-IUD depending on the patient satisfaction for three to 108 months, and 107 patients were followed-up but received no treatment. In conclusion, regression was achieved in 54% of the oral MPA group, 100% of the LNG-IUD group, and 50% of the non-treatment group. However, the objectivity of the study was compromised due to application of oral treatment in one group for only six months at most, while using LNG-IUD, known to reach markedly higher intrauterine concentrations, for a much longer time [14]. The success rate of 100% after the removal of the LNG-IUD at the end of the two years does not seem to match the data of Orbo et al. [14]. They have reported a response as low as 63% following the removal of the LNG-IUD in 22 of the 66 patients. In this study, the authors have preferred to use the WHO criteria for the classification of hyperplasia, and a homogeneous group of cases with hyperplasia without atypia was selected. Orbo et al. [14], however, have made a D-score-based classification. It is noteworthy in terms of the reliability of the classification that, in their study, there were 15 cases without atypia in the D score < 0 group which expresses a high malignant potential, and 33 cases with atypia in the D score > 1 group, which expresses a low malignant potential [14]. In addition, Wheeler has stated the cytologic atypia to be the most important prognostic factor in endometrial hyperplasia [15]. The treatment groups are highly variable and multicentered in Orbo’s study. The intrauterine release of LNG-IUD is very variable, changing from 3 to 108 months, with a very broad scale of follow-up, varying from 58 to 106 months [14]. On the other hand, the present study was more homogenous being performed in a single-center with a standard duration of application of LNG-IUD, and a 24-month follow-up for all patients. Vereide et al. applied oral MPA ten mg/day in 29 patients and LNG-IUD in 21 patients for three months; the biopsies obtained from the patients at three months follow-up displayed a success rate of 51.7% for the oral MPA group and 100% for the LNG-IUD group. In their study, although the treatment durations were the same, the follow-up period appears to be brief [9]. In the retrospective study of Buttini et al. [16], in a group of patients with hyperplasia without atypia, 22 cases were treated with LNG-IUD, and ten cases with oral progestin. A success rate of 100% was reported in the group with LNG-IUD in situ, whereas the group with oral progestin had a success rate of 70% [16]. In their retrospective study, Clark et al. [17] have reported that the cases with atypical hyperplasia were often treated by hysterectomy, and there was no consensus on how to approach patients without atypia. Although there was no clear data about the duration of the progestrone therapy, Randall et al. reported that minimum time span was nine months [7]. Wheeler has noted that, in the presence of atypia, there is a lower rate of regression and higher recurrence rates after discontinuation of the treatment [15].

In order to have an objective comparison, the oral MPA therapy was intended to be given for three or six months without interruption in beginning of the study. However, due to the risk of the side-effects of MPA and the absence of previously issued studies in which MPA was given in a similar way, this proposal was not approved by the Ethics Committee, and eventually the classical MPA treatment of ten days per month was given.

In the present study, except comparing the success of oral and intrauterine progesterone therapies, the authors aimed to determine the minimum duration of LNG-IUD treatment for successful outcomes, which appears to be a popular option in women with a wish for childbearing. Thus, each patient was followed-up for a period of two years. In relatively young patients who might have a wish...
for childbearing, the minimum duration of therapy that would be successful has not been studied by other authors. Therefore, one of the two objectives in this study was to determine the minimum amount of time required to achieve successful outcomes by LNG-IUD treatment.

Baseline and postoperative histopathologic evaluations on pipelle samples of 12 patients who underwent hysterectomy were performed by a single physician (KD) and evaluated by another single experienced cytopathologist (AB). Baseline and postoperative histopathologic evaluations of these 12 patients were found to be completely consistent. Since some cases of hyperplasia without atypia can resolve without any treatment, the absence of a third group receiving no therapy which would allow to investigate the spontaneous regression rates in people undergoing no treatment, can be recognized as a limitation of this study. Bearing this weakness in mind, the authors decided to avoid such a non-treatment group due to ethical concerns. Nevertheless, Orbo et al. observed that the regression rates of the non-treatment group and the standard low-dose (ten days a month, ten mg/day) MPA group were similar [14].

The success rates at two-years follow-up were 84% in the three-month LNG-IUD group and 50% in the three-month oral MPA group, whereas 100% in the six-month LNG-IUD group and 64% in the six-month oral MPA group. Regression rates were found to be statistically significantly higher in the LNG-IUD groups than in the oral MPA groups. Two recent studies have reported the regression rates of LNG-IUD therapy in hyperplasia without atypia as 92% (88/96) and 100% (12/12), which are consistent with the results of the present study [18, 19].

Since this study group consisted of patients that could have a wish for childbearing, they were aged below 50 years. One patient in Group 3 became pregnant 27 months after the removal of LNG-IUD and successfully gave birth with cesarean section four months prior. Moreover, one patient from Group 1 and another from Group 2 became pregnant two and three years after the treatment, respectively; the first patient ended her pregnancy by her own will and the latter experienced an abortion during the third trimester.

Conclusion

Previous oral progesterone and hysterectomy were the most common treatment modalities of endometrial hyperplasia, then LNG-IUD replaced the oral progesterone therapy and has become the most preferred alternative management to hysterectomy. The authors believe that LNG-IUD can be preferred as a safe and effective treatment of women for childbearing, particularly in cases without atypia, and that pregnancy can be planned after a six-month period of treatment.

References


Ondansetron or metoclopramide? Which is more effective in severe nausea and vomiting of pregnancy? A randomized trial double-blind study

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Introduction

Nausea and vomiting of pregnancy (NVP) is a common condition which involves about 80% of women during pregnancy [1]. The most severe form is known as hyperemesis gravidarum (HG), which widely is used characterized by “intractable vomiting associated with weight loss of more than 5% of prepregnancy weight, dehydration, ketosis, and electrolyte imbalances which may lead to hospitalization”. HG is estimated to occur in 0.5%-2% of pregnancies. The patients are more likely to be non-white and younger than 30 years [2].

Although NVP can be divided into three categories; mild, moderate, and severe, severity of vomiting may not adequately reflect the problems caused by pregnancy [3]. Physical and psychological effects of NVP often lead to feelings of anxiety and concerns about the impact on the fetus. As well as unfavorable effects on family relationships, it has undesirable consequences on a women’s job efficiency, as 47% out of employed women who suffer from NVP feel that their work efficiency is reduced [3]; also 35% of work hours are wasted (mean of 62 work-hours/month) [3] and 25% also have difficulty with household chores (mean of 32 work-hours/month) for each woman [1-4].

NVP is also considered as one of the reasons for termination of pregnancy [5]. It should not be surprising as it has been observed that some pregnant women experience severe nausea which is comparable with nausea in cancer patients after chemotherapy [6]. Each year a significant number of women are hospitalized due to NVP (14 admissions in 1,000 births) [7], so early diagnosis and proper treatment as healthcare management have a significant impact on quality of life during pregnancy.

The pathogenesis of NVP is not well known and seems to be multifactorial. Other causes of nausea and vomiting should be ruled out such as gastrointestinal, urogenital, and cerebral nerve system diseases as well as metabolic and toxic elements. Idiopathic NVP should be differentiated from the diseases which are associated with hydatidiform mole and multiple pregnancies [2].

The treatment of nausea and vomiting during pregnancy is approximately unendurable and miscellaneous types of treatments have been used so far.

We previously studied the effect of ginger (in biscuit form) as a nonpharmacological (herbal remedy) approach to nausea and vomiting in early pregnancy and found it was effective for relieving nausea and to some extent vomiting [8].

Within the antiemetic drugs, prochlorperazine, promethazine, metoclopramide, and pyridoxine (B6) have often been used as the first-line therapy [9]. Antiemetic effects of metoclopramide (MET) are a result of its anti-dopaminergic and likely the prokinetic function [10, 11]. A query revealed MET is effective in NVP and HG, with a good balance of efficacy and tolerability [12]. The newer treatment regimens of ondansetron (OND) or steroid-compounds have been considered as the first-line treatment while other treatments lead to failure. OND is a 5-hydroxytryptamine3 (5HT3) antagonist receptor that influences the central and peripheral nerves and reduces the activity of the vagus nerve which can stimulate the vomiting center in the medulla oblongata.

Summary

Background: Nausea and vomiting of pregnancy (NVP) are seen in 50-80% of pregnancies. However, in severe NVP, called hyperemesis gravidarum (HG), medical therapy to reduce nausea and vomiting is inevitable and ondansetron (OND) as an effective drug has recently been proposed. This study evaluated the effectiveness of OND versus metoclopramide (MET) in the treatment of HG. Methods: In this clinical trial study, 83 pregnant women with HG were enrolled in 2011-2012 and randomly divided in two groups. The first group received oral administration of MET and the second group was treated with OND for two weeks. Severity of nausea and vomiting were evaluated according to visual analogue scale (VAS) criteria. Data analysis was done by χ², Fisher exact test and Student’s t-test. Results: Comparison of the trend of change of vomiting in the two groups during the 14-day treatment showed the OND group had significantly lower vomiting scores versus the MET group (p = 0.042), while there was no significant difference in the trend of nausea. Conclusion: OND has a more favorable effect in controlling severe vomiting.

Key words: Metoclopramide; Ondansetron; Nausea; Vomiting; Pregnancy.
Its other effect includes blocking serotonin receptors in the chemoreceptor trigger zone (CTZ). It seems this drug is more efficient with minimum side-effects than previous antiemetic drugs (without drowsiness or extrapiramidal complications) [13]. One study examined treatment outcomes in women with severe nausea and vomiting of pregnancy receiving outpatient nursing support and either subcutaneous metoclopramide or subcutaneous ondansetron via a microinfusion pump and concluded treatment with either metoclopramide or ondansetron resulted in significant improvement of NVP symptoms with half the women showing a reduction in severe symptoms to moderate or mild symptoms within three days of treatment initiation. Alteration in treatment was significantly greater in patients initially prescribed metoclopramide [14].

Nausea and vomiting, especially in its severe forms, may reduce the quality of life of a pregnant woman, and information about OND in the pregnancy is limited [15], which is why its management is of interest [2]. Moreover, OND is not used for treatment of HG in our center, thus we decided to evaluate the effect of this medicine in the management of HG.

Materials and Methods

This randomized clinical trial double-blind study was done on 83 pregnant women with HG who were referred to the Ruhani Hospital of Babol University of Medical Science in the north of Iran from June 2011 to March 2012. The study was approved by the ethical committee of Babol University of Medical Science.

Inclusion criteria included hyperemesis pregnant women aged 18-35 years with primary or secondary pregnancy, gestational age less than 16 weeks, vomiting three times a day with weight loss more than 3 kg, and presence of ketonuria [2].

Patients with thyroid and gastrointestinal disease, hydatidiform mole, and multiple pregnancies were excluded from the study [16]. Gestational age less than 16 weeks was confirmed according to the patient’s last menstrual period and ultrasonography. All eligible patients signed an informed consent to enter the study.

After assessment of eligibility and recruitment before the intervention, patients were randomly allocated to receive one or another of the alternative treatments under study. A computer-generated randomization schedule was used and investigators and participants were all blinded to treatment arm assignments.

Unblinding took place after all participants had returned the final day of receiving medicine and a final letter was sent to them explaining which treatment arm they were in along with the preliminary study results. Both groups were matched for weight and age.

It is notable that none of the patients has used antiemetic medicines two weeks before the study. Also, at the onset of the study, the two groups were in a similar status for nausea and vomiting according to VAS criteria. All processes of the study were described to the patients. Subjects graded the severity of nausea by themselves according to VAS criteria and recorded the number of vomiting episodes in the last 24 hours before treatment.

The patients were randomly divided in two groups: 1: metoclopramide tablets, 10 mg, TDS, Hakim Pharmaceutical Co, Tehran, Iran. 2: ondansetron hydrochloride tablets, 4 mg, TDS, Chemie Pharmaceutical Co. Tehran, Iran) by a study coordinator who also encoded the drugs with matching random numbers.

All patients were evaluated as responding to treatment within two weeks according to VAS. Subjects graded the severity of their nausea and recorded the number of vomiting episodes in the last 24 hours before treatment and again during treatment days by themselves. Nausea is a subjective symptom, which is why VAS was used to quantify the changes in its severity [17, 18].

For VAS criteria, patients recorded the grade of severity of nausea on their first visit over the previous 24 hours by marking an “X” corresponding to their perceived states on a 10 cm vertical line, ranging from 0 (no nausea) to 10 (severe nausea).

On the following 14 days, recording the severity of nausea was done daily at bed time. The subjects also recorded the number of vomiting episodes in the last 24 hours before treatment, and then during the 14-day treatment. All patients received the medicine three times daily over a week. After one week the dose was gradually reduced and discontinued as follows: twice/days for three days, once/day for four days within the final week. The

| Table 1. — Sevirty of vomiting in the two groups within treatment days. |
|----------------------|----------------------|----------------------|----------------------|
| Treatment days       | Severity of vomiting (mean ± SD) | p value |
| Ondansetron          | Metoclopromide        |          |
| 1                    | 6.7 ± 3.1             | 5.1 ± 4.1           | 0.06 |
| 2                    | 6.0 ± 3.2             | 3.7 ± 3.8           | 0.006 |
| 3                    | 5.3 ± 3.3             | 3.2 ± 3.4           | 0.006 |
| 4                    | 5 ± 3.1               | 3.3 ± 3             | 0.013* |
| 5                    | 5.1 ± 3               | 3 ± 3.1             | 0.011 |
| 6                    | 3.8 ± 2.9             | 2.5 ± 2.6           | 0.047 |
| 7                    | 3.7 ± 2.8             | 2.7 ± 3.2           | 0.010 |
| 8                    | 3.1 ± 4.2             | 2.8 ± 3.4           | 0.028 |
| 9                    | 3.0 ± 3.7             | 2.9 ± 3.2           | 0.06 |
| 10                   | 3.1 ± 3.5             | 3.3 ± 3.3           | 0.36 |
| 11                   | 2.7 ± 3.2             | 2.8 ± 2.7           | 0.09 |
| 12                   | 6.9 ± 3.4             | 2.9 ± 2.5           | 0.10 |
| 13                   | 3.2 ± 3.3             | 2.8 ± 3.2           | 0.07 |
| 14                   | 2.9 ± 3.1             | 2.9 ± 2.4           | 0.10 |

*: Significant; p < 0.05.

| Table 2. — Severity of nausea in the two groups within treatment days. |
|----------------------|----------------------|----------------------|----------------------|
| Treatment days       | Severity of vomiting (mean ± SD) | p value |
| Ondansetron          | Metoclopromide        |          |
| 1                    | 6.8 ± 3.2             | 7.4 ± 2.8           | 0.39 |
| 2                    | 5.4 ± 3.2             | 6.7 ± 3.0           | 0.068 |
| 3                    | 5.4 ± 2.9             | 6.0 ± 2.9           | 0.024* |
| 4                    | 4.1 ± 2.9             | 5.7 ± 2.8           | 0.023* |
| 5                    | 4.1 ± 2.8             | 4.8 ± 2.5           | 0.32 |
| 6                    | 3.7 ± 2.7             | 4.3 ± 3.0           | 0.54 |
| 7                    | 3.7 ± 2.7             | 4.3 ± 2.8           | 0.25 |
| 8                    | 3.4 ± 2.8             | 4.2 ± 3.1           | 0.22 |
| 9                    | 3.2 ± 2.9             | 3.7 ± 3.0           | 0.52 |
| 10                   | 3.3 ± 3.3             | 3.5 ± 3.1           | 0.76 |
| 11                   | 2.7 ± 2.8             | 3.2 ± 2.7           | 0.53 |
| 12                   | 2.5 ± 2.9             | 3.4 ± 6.9           | 0.10 |
| 13                   | 2.2 ± 2.8             | 3.3 ± 3.2           | 0.12 |
| 14                   | 2.4 ± 2.9             | 3.1 ± 2.9           | 0.32 |

*: Significant; p < 0.05.

| Table 3. — Severity of nausea and vomiting in the first and second days one week after treatment in the groups under study. |
|----------------------|----------------------|----------------------|----------------------|
| Treatment days       | Severity of vomiting (mean ± SD) | p value |
| Ondansetron          | Metoclopromide        |          |
| Severity of nausea   | Day 1                 | 5.3 ± 3.2           | 5.7 ± 2.6           | 0.53 |
| (mean ± SD)          | Day 2                 | 3.4 ± 5.2           | 5.1 ± 3.4           | 0.87 |
| Severity of vomiting | Day 1                 | 4.6 ± 3.4           | 5.2 ± 3.1           | 0.42 |
| (mean ± SD)          | Day 2                 | 4.8 ± 3.5           | 4.7 ± 3.5           | 0.85 |
Ondansetron or metoclopramide? Which is more effective in severe nausea and vomiting of pregnancy? A randomized trial etc.

single dose was totally stopped at the end of the second week). All patients’ symptoms were evaluated within the first two days one week after stopping the medicine by the VAS criteria. After stopping the oral treatment, response to treatment was assessed, and if no improvement was observed in symptoms, the treatment protocol was revised according to the patient’s condition. Data of the two groups were collected and entered using statistical software SPSS18 and analyzed by central statistical indicators: t-test, Anova, and chi-square tests; \( p < 0.05 \) was considered significant.

### Results

Eighty-three patients were included in the study (41\% (34) in the MET group and 59\% (49) in the OND group). The mean age of the MET group was 25.2 ± 4.9 years and the mean age of the OND group was 25.3 ± 5.5 years. At the onset of the study, the mean weight was 63.9 ± 11.2 kg. Both groups were matched for age and weight, and the mean weight loss was 2.4 ± 2.8 kg. The minimum gestational age was five weeks and the maximum was 16 weeks (mean 8.7 ± 2.6 weeks). No significant difference was shown between the two treatment groups for mean gestational age and no relationship was seen between maternal age and number of nausea and vomiting episodes between the groups in the study.

No significant difference was found between the gestational age and number of nausea and vomiting episodes prior to treatment. The severity of nausea in the OND group was significantly less on the third and fourth days of treatment versus the MET group (\( p = 0.024, \ p = 0.023 \)). Also, the number of vomiting episodes in the OND group were fewer than the MET group from the second to the eighth days (Table 1, 2). Tables 3 and 4 show the mean severity of nausea and number of vomiting episodes in the first and second days one week after discontinuing the treatment. There was no significant difference between the two treatment groups.

Comparison of the trend of change of the number of vomiting episodes in the two groups during the 14 days of treatment have shown that the OND group had a significantly lower vomiting score versus the MET group (\( p = 0.042 \)), while there was no significant difference in the trend of nausea (Figures 1, 2).

None of the patients showed any side-effects of the offered medicines. All mothers and infants were healthy at the time of birth.

### Discussion

Although the findings of the study did not indicate any total superiority in all treatment days (14 days) in favor of one of the enrolled medicines, but a relative advantage was shown in favor of OND for nausea and vomiting in the first days of treatment versus MET. It is noteworthy that the trend of reduction of intensive vomiting was higher in the OND group.

Dabbous and et al. conducted a study on 200 patients and compared the antiemetic effects of OND with MET and droperidol. The results showed that both OND and droperidol were more effective than MET and patients were more satisfied with OND due to the rapid influence and less drowsiness [19]. Afhami and et al. compared the impact of MET and OND at post strabismus surgery in children. Patients were divided into two groups (48 children in each group) and demographic, hemodynamic, and duration of anesthesia were matched. The results suggest that the two groups were comparable with each other in incidence and severity of nausea and vomiting [13].

Gupta et al. [19] compared the antiemetic effects of OND and MET with granisetron in 60 patients undergoing laparoscopic cholecystectomy. Results revealed that in the first 12 hours post-surgery, there were fewer nausea and vomiting episodes in the granisetron group versus the two other treatment groups.

However after 12 hours, there were no significant differences observed between these medicines [20]. Also, Kroobuaban et al. compared the effects of OND and MET to reduce nausea and vomiting post-gynecological surgery among 382 patients. In this study, the number of women who complained of post-surgical nausea and vomiting were fewer in patients who received 4 mg of OND versus those who received 10 mg of MET (47\% vs 60\%) [21].

There have not been many studies on the antiemetic effects of these drugs on gestational nausea and vomiting in pregnant women, and most studies have been conducted on the patients after surgery or after chemotherapy.

Many queries have been done on the effects of newer antinausea drugs like OND and granisetron compared with older medicines such as MET. In a few studies the antiemetic effects of metoclopramide were comparable with OND, but most researches indicate OND has a stronger effect. In a random control trial study, Sullivan...
et al. compared OND with promethazine in the treatment of HG. They divided 30 patients in two groups and concluded OND was as effective as promethazine, however with less drowsiness. The authors of this article suggested increasing the dose of OND or using continuous infusion improves the response to treatment [15].

Shings et al. reported on a pregnant woman whose three previous pregnancies had been terminated due to severe HG and increased liver enzymes. They also intended to terminate her present pregnancy due to severe HG again. At the beginning they prescribed OND for the patient and two days after treatment, the patient was able to start a normal diet. The patient occasionally used MET after discharge.

Termination of pregnancy was done due to premature rupture of membranes and repeat cesarean at 35 weeks of pregnancy. The mother and baby were healthy. Also, the morphology and growth of the baby were within normal range after one year [22].

Ghahiri and et al. conducted a clinical trial study similar to our research with 35 pregnant women (in the first trimester) in each group; they were prescribed OND and MET and evaluated within three weeks. They findings showed that there was no a significant difference between groups in the mean of nausea episodes during the three weeks of treatment, but comparing both groups revealed OND made the mean number of vomiting episodes significantly lower after one week [23], while in our study the influence of both drugs appeared within the first week. Apparently, our difference is based on our chosen criteria; we used VAS and they used the number of nausea or vomiting episodes. Moreover, we enrolled severe NVP patients whereas they selected mild or moderate NVP, patients.

It should be noted that we had a limitation in our study; pregnant women used antiemetic drugs or nonmedication herbal medicines at the onset of their nausea and vomiting episodes and we hardly found patients who had received no antiemetic medicine within two weeks before the beginning of our study.

A well-designed study is required focusing on nausea and vomiting during pregnancy to compare herbal and synthetic medicines.

Conclusion
Our results showed that OND was able to diminish vomiting treatment more rapidly than MET and may be used effectively in the treatment of vomiting during pregnancy instead of MET.

References
Liquid based cytology and HPV DNA testing in a Greek population compared to colposcopy and histology

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Introduction

Cervical cancer still remains a major health issue despite efforts made to reduce its incidence rates. Since 1999, human papilloma viruses (HPVs) are considered a necessary cause of invasive carcinoma development [1]. A significant problem in Greece is inadequate epidemiologic data of diseases such as cervical cancer, due to the fact that screening is opportunistic, based exclusively on self-motivation. Although Pap test is free of charge, the coverage rate of regular screening in urban areas is less than 30% [2]. Available data indicate that 550 new cases of cervical cancer per year occur in Greece [3]. Although vaccination for HPV has already been introduced in the national vaccination program, only 11% of the target population between 11 and 26 years of age has been vaccinated until now. Thus cervical cancer will, in all probability, remain a prevailing public health issue for the imminent future.

The purpose of the current study was to evaluate the accuracy of cytological findings from a large observational population sample in association with reflex DNA test, colposcopic estimation, and final histologic diagnosis. The rate of invasive carcinoma, both squamous cell and adenocarcinoma, is indicative of a largely unscreened population. In this study, the estimated overall prevalence of human papilloma virus (HPV) was 41.1%, with HPV positivity at 37.4% of cytologically normal women. HPV testing did not seem to improve sensitivity of cytology for atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL) cases in identifying CIN 2+ lesions, but outperformed cytology in detecting CIN3+ for cytological high-grade squamous intraepithelial lesion (HGSIL) cases. For HGSIL cases sensitivity of colposcopy for detecting CIN3+ was comparable to cytology.

Key words: Cervix; Liquid based cytology; HPV DNA; Arrays; Colposcopy; Histology.

Materials and Methods

Study population

This is a cross-sectional study concerning 3,000 women with a median age of 34.3 ± 11.9 years (range 18 to 65 years), examined from March 2006 to September 2008. The population was consecutively recruited from the Third Department of Obstetrics and Gynaecology at the “Attikon” University Hospital.

The study population originated from Western Athens covering almost 0.1% of the capital’s population of reproductive and post-menopausal aged women. All women proceeded voluntarily to the outpatient clinic for regular gynecological control and if they fulfilled the criteria of the protocol, they were enrolled in the study. Women with recent labor were excluded, while all participants signed an informed consent form. Research was performed with the approval of both the National and Kapodistrian University of Athens and the “Attikon” University Hospital Bioethics Committees.

Sample collection

Liquid based cytology (ThinPrep®) Pap tests were collected by means of a Broom’s-like brush. The PreservCyt® vials were addressed to the Department of Cytopathology, for preparation of thin-layer slides using the ThinPrep 2000 Automated Slide Processor according to the manufacturer’s instructions. Cytologic findings were interpreted according to the Bethesda classification system (TBS) into eight categories; NILM (negative for intraepithelial lesion or malignancy), ASC-US and ASC-H (atypical squamous cells of undetermined significance) and ASC-US (atypical squamous cells of unknown significance) or cannot exclude high SIL), LSIL and HSIL (low- or high-grade squamous intraepithelial lesion), SCC (squamous cell carcinoma) and AdenoCa (adenocarcinoma). ASCUS+ cytologic findings or positive HPV testing referred women for colposcopy; cervical biopsies were collected from colposcopically suspicious sites. In cases with no obvious abnormalities, at least three blind biopsies were taken. All women with indications consented to this procedure. Tissue fragments were fixed in a 10% buffered formalin solution and embedded into paraffin; four μm thick sections were stained with a standard haematoxylin/eosin (H&E) stain. The three-tiered cervical intraepithelial neoplasia (CIN) grading system was used for histological diagnosis.
HPV DNA detection

ThinPrep® samples were stored at 4°C before DNA extraction. Procedures took place in two physically separated areas: the pre-PCR area, where samples were prepared and DNA was extracted and the post-PCR area, where products were amplified and visualised, minimizing the possibility of sample contamination with previously amplified products. The commercially available kits Papillomavirus Clinical Arrays® (Genomica, Spain) and CLART® Human Papillomavirus 2 (Genomica, Spain) were used for HPV DNA extraction and genotyping. All samples were analysed for the presence of the following 35 HPV types which are divided, according to their oncogenic status, into two categories: low-risk: 6, 11,40, 42, 43, 44, 54,61,62,71,72,81, 83, 84 and 89 and high-risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 and 85 [4].

HPV DNA was amplified using biotinylated PGMY primers that target a 450 bp long fragment of the viral L1 gene. Detection of the amplified PCR product was performed using a low-density microarray, anchored in a two ml array tube that allowed simultaneous detection of 35 different HPV types and included controls to ensure a feasible assay. Results’ analysis was performed automatically.

Results

Cytology results and HPV detection

The study population originally included 3,000 women. 155 of these women (5.1%) were finally excluded due to lack of compliance or due to inadequate first sampling. Out of the final 2,845 samples, 2,442 had a negative (NILM) Pap smear (85.8%) whereas 403 (14.2%) were positive for cytological abnormalities of any kind according to TBS (Table 1). Among the 2,845 women included in the study population, HPV DNA test was positive in 1,234, i.e. an overall prevalence of the virus of 43.4%; HPV positivity was identified in 37.4% of NILM samples, 70.6% of ASCUS, 78.7% of LSIL samples, 100% of ASC-H, 93.8% of HSIL, 100% of AdenoCa, but only in 90% of the SCC.

Detection of genotypes among HPV positive women with NILM cytology identified a high-risk type in 81.5% of positive samples. Significantly, in 51.3% of these cases infection with multiple high-risk types was found, whereas 30.2% had a single high-risk type infection. The percentage of low-risk type infections (both single and multiple) was 18.5%.

Among HPV positive samples of ASCUS+ lesions, high-risk type infections were detected in 84.7% of ASCUS samples, 87.3% of LSIL samples, 95.6% of HSIL, 90% of AdenoCa, and 100% of ASC-H and SCC samples. The rate of multiple to single type infection was very close to 2:1 in all Bethesda categories, except for ASCUS (1:1) and SCC (1:2).

Colposcopical results

Among women with NILM cytological diagnosis, 914 tested positive for HPV and were therefore referred for colposcopy. Out of these, 867 (94.9%) had no colposcopic findings, whereas 31 (3.4%) had colposcopic findings compatible to LGSIL and only two (0.2%) were colposcopically evaluated as HGSIL. Colposcopy was inadequate in 14 cases. Results are summarized in Table 2.

All women with abnormal cytology were referred for colposcopy. From cases with LGSIL Pap test, 11.7% had an unremarkable colposcopy, 84.3% had colposcopic findings compatible with LGSIL, and 3.5% compatible with HGSIL. Women with HGSIL cytology had more characteristic findings at colposcopy and only 2.1% were found negative, whereas 70.7% had colposcopic findings compatible with as HSIL. Yet, 20.9% of cases with abnormal Pap test were colposcopically underestimated. Among women with ASCUS smears, 35.3% had no colposcopic indications of any abnormality, whereas 18.6% were estimated to have severe lesions. All women with ASC-H or SCC Pap test presented colposcopic findings. One case of AdenoCa was negative. Concerning cases

| Table 1. — ThinPrep diagnosis and biopsy results in relation with HPV DNA testing. |
| HPV/ | Cytology | Negative | Positive | High single | High multiple | Negative | Total |
| NILM | 1,528 | 914 (37.4) | 276 (30.2) | 469 (51.3) | 169 (18.5) | 2442 |
| ASC-US | 30 | 72 (70.6) | 23 (31.9) | 38 (52.8) | 11 (15.3) | 102 |
| LSIL | 49 | 181 (78.7) | 59 (32.6) | 99 (54.7) | 23 (12.7) | 250 |
| ASC-H | 0 | 3 (100) | 1 (33.3) | 2 (66.7) | 0 | 3 |
| HSIL | 3 | 45 (93.8) | 13 (28.9) | 30 (66.7) | 2 (4.4) | 48 |
| SCC | 1 | 9 (90) | 7 (77.8) | 2 (22.2) | 0 | 10 |
| AdenoCa | 0 | 100 (100) | 3 (30) | 6 (60) | 1 (10) | 10 |

| Table 2. — Correlation of cytology with colposcopic findings. |
| Colposcopy/ | Cytology | NILM | LSIL | HSIL | SCC | Inadequate |
| WNL | 914 | 867 (94.9) | 31 (3.4) | 2 (0.2) | 0 | 14 (1.5) |
| ASC-US | 102 | 36 (35.3) | 45 (44.1) | 19 (18.6) | 0 | 2 (2) |
| LSIL | 230 | 27 (11.7) | 194 (84.3) | 8 (3.5) | 0 | 1(0.5) |
| ASC-H | 3 | 0 | 3 (100) | 0 | 0 |
| HSIL | 48 | 1 (2.1) | 9 (18.8) | 34 (70.7) | 3 (6.3) | 1 (2.1) |
| SCC | 10 | 0 | 2 (20) | 8 (80) | 0 |
| AdenoCa | 10 | 5 (50) | 1 (10) | 3 (30) | 1 (10) |
| Total | 1317 | 936 | 280 | 71 | 12 | 18 |

| Table 3. — Correlation of cytological and histological diagnoses. |
| Colposcopy/ | Cytology | NILM | CIN I | CIN II | CIN III | SCC | AdenoCa |
| WNL | 914 | 862 (94.3) | 50 (5.5) | 1 (0.1) | 1 (0.1) | 0 | 0 |
| ASC-US | 102 | 7 (6.9) | 92 (90.2) | 3 (2.9) | 0 | 0 |
| LSIL | 230 | 23 (10) | 173 (75.2) | 30 (13) | 3 (1.3) | 0 | 1 (0.4) |
| ASC-H | 3 | 0 | 1 (33.3) | 1 (33.3) | 1 (33.3) | 0 |
| HSIL | 48 | 1 (2.1) | 5 (10.4) | 26 (54.1) | 14 (29.2) | 2 (4.2) | 0 |
| SCC | 10 | 0 | 0 | 0 | 10 (100) | 0 |
| AdenoCa | 10 | 0 | 0 | 3 (30) | 1 (10) | 6 (60) |
| Total | 1317 | 893 | 321 | 61 | 22 | 13 | 7 |
with cytologic diagnosis of AdenoCa. 83.3% had colposcopic findings compatible with LGSIL or HGSIL.

It is noteworthy that 18.8% of cytologically HSIL women were colposcopied as of lower significance abnormality and even one case as negative (2.1%). Almost one-third of high-grade lesions were not identified during colposcopy, although performed by an expert clinician.

**Histological results**

Comparison of cytologic and histological results is presented at Table 3. Histologically-normal were 94.3% of the cytology NILM HPV-positive cases, 10% of the LSIL, 2.1% of the HSIL, and 6.9% of the ASCUS cases. None of the ASC-H, AIS, SCC, and AdenoCa were histological normal. Among cytological NILM samples, 5.5% had a biopsy diagnosis of CIN 1; one case was histologically diagnosed as CIN 2, and one as CIN 3.

Cytological LSIL cases had an underlying lesion of CIN 2 in 14.7% of cases, including one case of AdenoCa. The cytological diagnosis of HSIL was more efficient, when compared to the golden standard of histology, since there was only 12.5% of ≤ CIN 1. In ASCUS cases, 90.2% had a ≤ CIN 1 in biopsy. While concordance was found in 60% of AdenoNoCa, no case was lost, as the remaining were CIN3+.

Only one case (2.1%) of HSIL was histologically normal and five (10.4%) were CIN 1, forming the total percentage of those with lighter or no abnormality demonstrated by histology at 12.5%. The one HSIL case with normal histological diagnosis was the same one that was colposcopically negative.

Correlation of histology with HPV testing results revealed that 62.4% of the diagnosed as NILM samples were actually HPV-positive. In particular 89.9% had high-risk type infection, with an overbalance of multiple types versus single type infection. This phenomenon was identified also in all CIN lesions. While 100% of the tested samples with verified AdenoCa were HPV positive, especially with a high-risk single type infection, only 84.6% of the SCC was positive and with the same characteristics: high-risk single type infection.

The correlation of histology with colposcopy revealed an underestimation of the biopsy confirmed CIN 2 cases at 54.1%. There was a concordance of CIN 2+ histology and high-grade lesion colposcopically at 53%. Since there was a subsuming of all "suspicious HPV" histological diagnoses at the HPV category, as already mentioned, there were 22.2% of cases estimated as negative colposcopically. All tests’ performance is summarized in Table 4 compared to the golden standard of histology.

**Discussion**

In this study accuracy of cytological findings were evaluated by comparing cytological diagnoses along with reflex DNA testing, colposcopic examination, and final histologic diagnosis from samples obtained according to protocol. In the present study, the authors estimated the overall prevalence of HPV at 41.1%. Other studies in the Greek population demonstrated prevalence ranging from 2.5% up to 60% [2, 5-8] while in a more recent study, HPV was detected by consensus PCR in 31.3% of the samples [9]. As new typing methods increase the number of HPV types detected, it is perspective that more infections will be identified and the prevalence will augment.

HPV DNA was positive in 37.4% of cytologically normal women in agreement with some studies [6, 10]; yet, rates of HPV detection in such cases vary widely in literature ranging from 3% to 34.3% when consensus PCR had been used [11-15] and probably reflected the high analytical sensitivity of the detection method used. Although almost 80% of women have transient infections [16], since HPV infection precedes the development of SILs [17], women with normal cytology but HPV positive should be prospectively followed by their gynecologist and submitted to cytology and other testing where appropriate [18, 19]. In the vast majority of women with normal cytology, who were referred to colposcopy, no detectable lesion was identified; further supporting that HPV DNA testing cannot be used in screening due to its low PPV (Table 4). Cases histologically confirmed as CIN 1 but negative for HPV DNA could be the result of either viral clearance during the time window between cytology and histology, poor sampling during cytology testing, or loss if the L1 viral gene that is the target of the
molecular technique used at the present study, due to viral integration into the host genome [1]. HPV DNA negative squamous cell carcinomas may, also be related to full integration of HPV or to a “passenger effect” of the virus, where viral replication was inhibited in that specific genetic environment [20].

The reported results (79.4% HPV-DNA positive among cytologically abnormal samples) confirm the causal relationship between HPV infection and abnormal cytology, in concordance with most studies published so far, demonstrating an increase of HPV prevalence related to higher grading of squamous intraepithelial lesions [11, 21-24]. In the study population, 3.4% was diagnosed as ASC-US and 0.1% as ASC-H plus. ASC-H is reported in the literature in 0.7% - 0.6% of all Pap test results [25-27] while the mean frequency of detection of ASC-US in the USA is 4.7% of all smears [28]. The results in the present study seem to be in concordance with the literature estimating that the frequency of ASC-US should not exceed two to three times the frequency of LSIL [25, 29-35]. The authors must also mention that the number of ASC-US cases is significantly low for the “high-risk” study population evaluated in the current study, where cervical cancer incidence was 0.7%, rather than the 0.1% anticipated in the general population. Approximately 70% of the ASC-US smears and all 100% of the ASC-H were HPV positive. Colposcopy verified the presence of abnormalities in 62.7% and 100% respectively as did histology in 93.1% of ASCUS and 100% of ASC-H cases. Since sampling collection was performed by experienced gynecologists and examined by trained cytopathologists with at least five years experience in liquid based cytology (LBC), these results were more or less as anticipated. Yet, histologic results raised questions about screening intervals for these patients and about treatment options, since according to a meta-analysis, the absolute risk of underlying CIN2+ and CIN3+ among women with ASCUS is on average 9-10% and 4-5% respectively [36]. The ALTS study documented a cumulative risk of high-grade disease at 26.7% for women with HPV-positive ASCUS [37].

The use of HPV testing has been recommended for women with ASC-US [38]. HPV DNA testing seems to be more sensitive than colposcopy in ASCUS cases [39]. In this study, HPV testing did not seem to improve sensitivity of cytology in ASCUS cases in identifying severe lesions (Table 4). Keeping in mind that, although the HPV assay results were performed as quickly as possible, yet the interval of time may have altered the virus status and that may have affected the test performance. The ASCC and ACOG management guidelines for women with HPV-positive ASC-US recommend immediate referral to colposcopy [21-22, 29, 40]. Literature reports [21-22, 39, 41-42] that 20-60% of ASCUS cases are associated with CIN colposcopic diagnosis, yet among them, 70% are CIN 1. The results in this study agree with the literature, since in 62.7% of all ASCUS cases the presence of a lesion was colposcopically identified. However, the results showed that for such cases colposcopy did not seem to improve sensitivity of cytology in identifying CIN2 and CIN3 lesions with specificity. For women with cytological ASC-H diagnosis, the association with high-risk HPV was suggested to carry a higher risk of CIN 2+ in 40% [43], yet in 66% in this study. The detection of HPV types among women with ASC-H diagnosis seems to improve sensitivity of cytology for detecting both CIN2 and CIN3 cases. This clue, along with that colposcopy, also seems to be a more sensitive method, and must be taken into account in order to manage women with ASC-H cytology.

In cytological LSIL cases, neither HPV testing, nor colposcopy outperformed cytology that had comparable, if not better results. The pooled results of a recent meta-analysis indicated that reflex HPV testing is insufficiently discriminative in case of LSIL, as the large majority of LSIL cases were high-risk HPV positive [44]. Yet, the cumulative risks of CIN2+ and CIN3+ among HPV positive women with LSIL cytology resulted in 30.3% and 17.2% respectively according to the recent TOMBOLA study [45]. These women were set to a more extensive follow-up during second and third round of this study, by protocol. The 14.7% of LGSIL cases that actually had an underlying CIN 2+ lesion during the first round of this study, is estimated to decrease at 12 months and even more at 24 months [46, 47]. It must be noted that for some cases there was a significant time delay between cytology and referral to colposcopy, and some lesions may have regressed.

Sensitivity of HPV testing for detecting CIN3+ for cytological HSIL cases outperformed cytology, yet with significantly lower specificity. When the HSIL+ cytological lesion cutoff point was used, sensitivity of colposcopy for detecting CIN3+ was comparable to cytology, as shown by others [48].

The rate of invasive carcinoma discovered in this study is indicative of a largely unscreened population. Among cytologically SCC samples, 77.8% had single high-risk type infections. This fact seems to be in concordance with the hypothesis that a certain type may become dominant over others as the disease progresses [49] and that cervical neoplasia is a result of clonal expansion of a cell infected with a single type HPV [50]. Two out of 13 SCC histologically-verified cases tested negative for HPV. For these cases either cervical cancer may have been caused by a different mechanism, or an HPV type not detected by the methods used, or the HPV causing the cervical carcinogenesis may have been lost, since some carcinogenic types may have presented only as passengers [51].

A cytological result of adenocarcinoma in situ (AIS), as demonstrated by several studies, is associated with 48-69% risk of biopsy-confirmed AIS and 38% risk of invasive adenocarcinoma. In the presented study, there seems to be an agreement with these findings, although the small number of cases should be kept in mind. All of the cases tested HPV positive, yet the 2001 Consensus Conference concluded that there was insufficient data to allow an assessment of the role of HPV DNA testing in the management of AGC and AIS [52]. The results indi-
Liquid based cytology and HPV DNA testing in a Greek population compared to colposcopy and histology

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References


cated also the anticipated: for such cases, colposcopy is rather difficult to set a diagnosis.

The overall results, as presented in Table 4, coincide with TBS 2001 recommendations, and indicate that when cytological diagnosis stands on a very good level, immediate referral to colposcopy is acceptable. Given the extremely high-negative predictive value of Pap test, the actual possibility of a CIN 2+ lesion underlying a NILM diagnosis is quite small. If the cut-off cytological diagnosis for referral is set at LSIL+ a significant gain in specificity with a minor drop of sensitivity is observed, compared to ASC-US+ with an end-point of CIN 2+. On the other hand, by setting the ASC-H+ diagnosis as the cut-off, a great gain in PPV, NPV and specificity is observed, while sensitivity is cut down. Combining results from Table 4 indicate that with an ASC-H+ diagnosis and a positive HPV DNA test, colposcopists must be extremely careful because there is a great possibility of an underlying severe lesion. On the other hand, a NILM cytology combined with a negative colposcopy, has an underlying lesion in only 0.8%. Since in almost 40% of such cases HPV DNA test is positive, this particular examination is not cost-effective.

The limitation of a population that is not systematically screened, but is consecutively enrolled is of course recognized. Despite the excellent results of cytology in this study, it is well-known that screening for cytological changes may have limited sensitivity and findings are not always reproducible [51]. In Greece the financial value of colposcopy is lower than HPV testing. Although sensitivity and specificity of colposcopy are moderate, negative predictive value is exceptionally good. From both the clinician and patient perspectives, the predictive values are the most important parameters. Positive predictive value is accepted to be very low, since the visual changes caused by HPV and identified by colposcopy are quite common. Thus, the high-negative predictive value reassures that women tested negative could be examined periodically by test Pap and colposcopy with larger yet safer time intervals [53]. Moreover, molecular HPV testing should not be introduced without careful planning; results of such testing should be communicated and explained appropriately in the context of prevalence of the disease.
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The role of mini laparotomy in patients with uterine myomas

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Summary

Aim: The aim of this study was to evaluate the therapeutic effectiveness of myomectomy by mini laparotomy in patients with subserosal and/or intramural uterine myomas. Materials and Methods: Between January 2002 and December 2008, 83 women with symptomatic uterine myomas were referred to the Second Department of Gynecology of St. Savvas Anticancer Oncologic Hospital of Athens. The study included women with subserosal and/or intramural uterine myomas with a maximum diameter of ten cm. All patients underwent myomectomy by mini laparotomy. Results: The median age of the patients was 36.8 years (range 19 - 43). The median number of the removed uterine myomas was 3.1 (range 1 - 12) and the median operative time was 98 minutes (range 47 - 170). All patients were mobilized within the first 24 hours and the median time of postoperative ileus was 1.6 days (range 1 - 3). The median hospital stay was 44 hours (range 30 - 120). There were no serious intraoperative or early postoperative complications. Conversion to laparotomy was performed only in four cases (4.82%), but none of the patients underwent emergency hysterectomy. During a mean follow up of 38 months, no recurrences of uterine myomas in the study population were observed. Conclusion: Mini laparotomic myomectomy is a safe and effective minimally invasive method alternative to laparoscopic myomectomy for patients with subserosal and/or intramural uterine myomas.

Key words: Myomectomy; Mini laparotomy; Mini laparotomic myomectomy; Uterine myomas.

Introduction

Uterine myomas are benign, hormone-sensitive, fibromuscular tumors that are diagnosed in about 25% to 40% of women during their reproductive age [1]. Uterine myomas may be asymptomatic or they can cause abnormal uterine bleeding, pelvic pain, pressure complaints, pregnancy-related complications, and infertility [2].

The management of women with uterine myomas remains controversial. Various treatment protocols use medical treatment (progestins, gonadotropin releasing hormone (GnRH) analogues), radiological treatment (uterine artery embolization) or surgical intervention (myomectomy, hysterectomy) [3-7]. Recent advances in the nonsurgical management of uterine myomas have shown promising results by simplifying or eliminating the need for surgical intervention, but they are inappropriate for infertile women and for women wanting to preserve future childbearing capability [8]. For these women, myomectomy is the treatment of choice [7, 8].

Classical laparotomic myomectomy is associated with significant morbidity including excessive blood loss, infection, and postoperative adhesions [9]. Laparoscopic myomectomy is an alternative, with fewer complications, shortened hospital stay, and less disability [6]. However, laparoscopic approach is a tedious operation especially in intramural uterine myomas and requires skilled suturing [10, 11]. Mini laparotomy represents a minimally invasive technique which can be used also in uterine myomas [7, 12].

The aim of this study was to evaluate the therapeutic effectiveness of myomectomy by mini laparotomy in patients with subserosal and/or intramural uterine myomas.

Materials and Methods

Between January 2002 and December 2008, 83 women with symptomatic uterine myomas were referred to the Second Department of Gynecology of St. Savvas Anticancer Oncologic Hospital of Athens. The study included women with subserosal and/or intramural uterine myomas with a maximum diameter of ten cm. All patients underwent myomectomy by mini laparotomy.

Preoperatively, all patients underwent transvaginal ultrasonography (TVUS) to evaluate the presence or absence of associated pelvic disease and to determine dimensions, number, and location of uterine myomas. Bowel preparation and antithrombotic prophylaxis were performed, and short-term intraoperative prophylactic antibiotic therapy with a second generation cephalosporin was administered to all patients. Postoperatively, two more dosages of antibiotic therapy were administered. When the resulting defect was extensive, metronidazole was added.

The surgical procedure was performed with the patients under general anesthesia with their bladder catheterized. The operation commenced with a four to six cm transverse skin incision, two cm above the pubis. To avoid accidental lengthening of the incision, it was sutured at both ends. The abdominal fascia was opened transversely two cm above skin incision at a total length of six to eight cm. The abdominal muscle was opened longitudinally on the midline and entered into the parietal peritoneum. Then an elastic wound protector/retractor of four to six cm was used. An atraumatic uterine manipulator was also used in order to elevate the uterus toward the incision without causing mechanical damage.
The surgical technique was basically the same as in laparotomy, but the surgeon had to perfect manual skills in working with the instruments in a vertical position, because their wide inclination was not possible. Myomectomy and uterus reconstruction was performed directly outside of the peritoneum.

A linear uterine incision, as small as possible, was made on the most prominent part of the myoma. If there were multiple myomas, a strategic incision site was attempted through which most of the myomas were removed. Careful palpation of the uterus allowed identification of additional myomas. Mini laparotomy was closed in separate layers.

The incision was not possible. Myomectomy and uterus reconstruction was performed only in four cases (4.82%). None of the patients required emergency hysterectomy (n = 83).

Table 1. — Patient characteristics (n = 83).

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<td>BMI (kg/m²)</td>
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<tr>
<td>Number of uterine myomas</td>
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<td>(1 - 12)</td>
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<td>Largest uterine myoma (cm)</td>
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<td>(4 - 10)</td>
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Table 2. — Patient operative parameters (n = 83).

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</thead>
<tbody>
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<td>Median operative time (min)</td>
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<td>(47 - 170)</td>
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<td>Decrease in Hb levels (g/dl)</td>
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<td>(0.3 - 4.4)</td>
</tr>
<tr>
<td>Time of postoperative ileus (days)</td>
<td>1.6</td>
<td>(1 - 3)</td>
</tr>
<tr>
<td>Postoperative hospital stay (hours)</td>
<td>44</td>
<td>(30 - 120)</td>
</tr>
</tbody>
</table>

Table 3. — Intraoperative and postoperative problems encountered (n = 83).

<table>
<thead>
<tr>
<th>Problem</th>
<th>Yes (% of cases)</th>
<th>No (% of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postoperative fever &gt; 38°C</td>
<td>4 (4.82%)</td>
<td>79 (95.18%)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>1 (1.2%)</td>
<td>82 (98.8%)</td>
</tr>
<tr>
<td>Conversion to laparotomy</td>
<td>4 (4.82%)</td>
<td>79 (95.18%)</td>
</tr>
<tr>
<td>Emergency hysterectomy</td>
<td>0 (0%)</td>
<td>83 (100%)</td>
</tr>
<tr>
<td>Serious intraoperative &amp; early postoperative complications</td>
<td>0 (0%)</td>
<td>83 (100%)</td>
</tr>
</tbody>
</table>

Results

The median age of the patients was 36.8 years (range 19 - 43) and the median body mass index (BMI) was 26.7 kg/m² (range 19 - 31). The median number of the removed uterine myomas was 3.1 (range 1 - 12). The median size of the largest uterine myoma was 5.7 cm (range 4 - 10). In this study, 36 patients received GnRH analogues preoperatively, because they had increased uterine size and/or low hemoglobin (Hb) levels. Patient characteristics are shown in Table 1.

The median operative time was 98 minutes (range 47 - 170) and the median decrease in Hb levels was 2.6 g/dl (range 0.3 - 4.4). All patients mobilized within the first 24 hours and the median time of postoperative ileus was 1.6 days (range 1 - 3). The median hospital stay was 44 hours (range 30 - 120). The operative parameters are shown in Table 2.

Although body temperature > 37°C was observed in all patients, postoperative fever > 38°C was observed only in four cases (4.82%). In this study, there were no serious intraoperative or early postoperative complications. Intraoperative blood transfusion was performed only in one case (1.2%). Conversion to laparotomy was performed only in four cases (4.82%). None of the patients underwent emergency hysterectomy. The intraoperative and postoperative problems are shown in Table 3.

All patients had significantly less postoperative pain (less postoperative use of continuous pain control anesthesia without increase in accumulated dose of pethidine). Furthermore, they had no request of analgesics after 48 hours postoperatively. Almost all patients returned to normal activity two weeks postoperatively.

All patients were evaluated postoperatively with gynecologic examination and TVUS. During a mean follow-up of 38 months (range 6 - 72), there were no recurrences of uterine myomas or symptoms in the study population. There were also no recurrences of uterine myomas in the subgroup of women pretreated with GnRH analogues during the same follow-up period.

Discussion

Certainly for infertile women and for women desiring to preserve future childbearing capability, myomectomy remains the treatment of choice [7, 8]. Classical laparotomic myomectomy is associated with significant morbidity, including excessive blood loss, infection, and postoperative adhesions [9]. Minimally invasive techniques (laparoscopy and mini laparotomy) offer operative accuracy and early postoperative advantages documented in the treatment of benign and malignant gynecologic diseases [12-14]. They are a valid alternative to classical laparotomic myomectomy, with comparable earlier recovery, shortened hospital stay, long-term outcomes, and better quality of life [12].

However, many gynecologists are not skilled laparoscopists to perform laparoscopic myomectomy and uterine repair [15]. In order to maintain the efficacy of uterine repair and to reduce the clinical impact of laparotomic myomectomy, mini laparotomic myomectomy was proposed as an alternative to laparoscopic myomectomy [15, 16]. Mini laparotomy allows simple and less traumatic access to the pelvis, regardless of uterine size and/or previous abdominal surgeries [13]. It fulfills the criteria of
minimally invasive surgery, is less expensive, without compromising postoperative recovery of patients [17]. Also, it has less contraindications due to patient’s clinical conditions [12]. Severe obesity represents the only factor statistically correlated with unsuccessful mini laparotomic surgery, especially in patients with BMI > 30 [7]. Considering the low extent of tissue trauma and the absence of retractors, mini laparotomy can elicit a neuroendocrine stress response less relevant to laparotomy and similar to laparoscopy [18].

Mini laparotomic myomectomy is technically less difficult to perform than laparoscopic myomectomy and gives the opportunity to seal the uterine defect properly and adequately, requiring less operative time and cost [12]. It is obvious that surgical technique in mini laparotomic myomectomy is basically the same as in classical laparotomic myomectomy [12]. In this study, the median operative time was 98 minutes (range 47 - 170) and the uterine defects were sealed with interrupted sutures.

Pretreatment with GnRH analogues before mini laparotomic myomectomy, remains controversial [7]. Preoperative use of GnRH analogues for three to four months: improves hematocrit levels, reduces myomas size, total uterine volume, and reduces intraoperative blood loss [19]. This is very important, especially for patients with anemia and/or large uterine myomas. In this study, 36 women were pretreated with GnRH analogues for three months due to anemia and/or large uterine myomas.

It is well known that intraoperative blood loss in myomectomy is correlated with preoperative uterine size, total weight of removed uterine myomas, and total operating time [20]. Certainly, preoperative use of GnRH analogues render mini laparotomic myomectomy technically easier and less time-consuming [7]. However uterine myomas become softer, and in some cases this can result in increased bleeding during operation [7]. Also, there may be an increased risk of recurrence, because small uterine myomas are recognized intraoperatively with difficulty [19]. In this study, no intraoperative difficulties were experienced, although 36 women were pretreated with GnRH analogues for three months. During a mean follow-up of 38 months, no recurrences of uterine myomas in the same subgroup of women pretreated with GnRH analogues were observed.

The greatest advantages of mini laparotomic myomectomy are: less intraoperative or early postoperative complications, low risk of conversion to laparotomy and low risk of recurrence compared with laparoscopic myomectomy [12, 21-23]. In this study, no serious intraoperative or early postoperative complications were shown and conversion to laparotomy occurred in only four cases. Also, there were no recurrences of uterine myomas, during a mean follow-up of 38 months. It is conceivable that the results regarding the recurrence rate of mini laparotomic myomectomy could be similar to those of laparoscopic myomectomy [12]. An obvious explanation is that with mini laparotomic approach, it is possible to palpate the uterus and recognize small intramural uterine myomas intraoperatively [12].

Other important advantages of mini laparotomic myomectomy are: significantly less postoperative pain (less postoperative use of continuous pain control anesthesia without increase in accumulated dose of pethidine) and better recovery (earlier mobilization, shorter time of postoperative ileus, and shorter hospital stay) [17]. In this study, all patients had significantly less postoperative pain and they had no request of analgesics 48 hours after surgery. Also, all patients mobilized within the first 24 hours, the median time of postoperative ileus was only 1.6 days (range 1 - 3) and the median hospital stay was only 44 hours (range 30 - 120). Almost all patients returned to normal activity two weeks postoperatively.

**Conclusion**

Mini laparotomic myomectomy is a safe and effective minimally invasive method alternative to laparoscopic myomectomy for patients with subserosal and/or intramural uterine myomas.

**References**


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Ankaferd blood stopper in episiotomy repair

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Zekai Tahir Burak Women’s Health Research and Education Hospital, Ankara (Turkey)

Summary

Background and Objective: Ankaferd blood stopper (ABS) is a new hemostatic agent that is licensed for external hemorrhages. ABS comprises of a standard mixture of Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, and Urtica dioica which has also been approved in Turkey for the management of bleeding. The authors, aim was to evaluate the efficacy of ABS spray in terms of blood loss during episiotomy repair. Materials and Methods: The authors included pregnant women with a term singleton fetus (37-40 wks) in a vertex position, who were at least 18-years-old, had delivered vaginally, and required a mediolateral episiotomy. The patients were randomly assigned to one of the two approaches: 20 (Group 1) to ABS and 20 (Group 2) to isotonic saline solution (0.9% NaCl). The authors applied 4 ml ABS spray solution (1 ml/puff X 4) or isotonic saline solution (0.9% NaCl) (4 ml) topically on a sponge applied on the episiotomy. The sponge was weighed before and after the episiotomy repair to determine the amount of bleeding. Hemoglobin values were also recorded on admission and 12 hours after delivery. Results: Both groups were similar in terms of maternal age, parity, body mass index and gestational age. The sponges weighed heavier in Group 2. Baseline hemoglobin values measured on admission showed no significant differences between the groups. Hemoglobin on the first postpartum day was significantly higher in the ABS group (p < 0.05). The operative time for episiotomy repair for the two groups was also statistically insignificant. The total amount of bleeding was significantly less in the ABS group. Conclusion: In this study group, the application of 4 ml of ABS instead of isotonic saline solution lessened bleeding.

Key words: Ankaferd; Episiotomy.

Introduction

Ankaferd blood stopper (ABS) is a standardized herbal extract obtained from five different plants Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, and Urtica dioica [1]. ABS has been approved in Turkey for the clinical management of external postsurgical and postdental surgical bleeding, but its mechanism of action remains unknown.

Numerous reports have been published regarding the application of ABS to control bleeding [2-7]. ABS represents a unique hemostatic effect by promoting the very rapid (< 1 second) formation of a protein network, which acts as an anchor for vital physiological erythrocyte aggregation, while covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets [1]. Exposure to ABS seems to provide tissue oxygenation as well as physiological hemostatic process without affecting any individual clotting factor [2-7].

Although the use of episiotomy is often debated, it remains the most common surgical procedure experienced by women [8]. The authors’ aim was to evaluate the efficacy of ABS spray in terms of blood loss during episiotomy repair.

Materials and Methods

This research was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Hospital and by the 7th Ethics Committee. Consent was obtained from all patients after full explanation of the procedure.

The authors included pregnant women with a term singleton fetus (37-40 wks) in a vertex position, who were at least 18-years-old, had delivered vaginally, and required a mediolateral episiotomy. The episiotomy was performed with scissors by the same obstetrician. It was defined as a six-cm incision at a 45-degree angle from the inferior portion of the hymeneal ring.

The authors excluded patients who were taking anticoagulation medications (warfarin, heparin, or enoxaparin), had systemic bleeding disorders (von Willebrand disease and hemophilia) or had systemic conditions that caused coagulopathies (liver disease), or had abnormal biochemical parameters. Patients were randomly assigned to one of the two approaches: 20 (Group 1) to ABS and 20 (Group 2) to isotonic saline solution (0.9% NaCl). A randomized number was assigned by using random allocation software.

ABS is available in spray form and is a registered product of a combination of plant extracts. ABS was obtained from Trend Teknoloji İlaç AS, Istanbul (Turkey) as a solution for direct application in the pharmaceutical form of spray.

The authors applied 4 ml of ABS spray solution (1 ml/puff X 4) or isotonic saline solution (0.9% NaCl) (4 ml) topically on a sponge applied on the episiotomy. Based on the manufacturer’s recommendation, the sponge was weighed before and after the episiotomy repair for ABS group of patients treated in this study, and another sponge was used to evaluate bleeding. The authors then weighed (scale SKS 4507 Simbo, made in P.R.C, 2009) the sponge after the procedure to determine the amount of bleeding. Hemoglobin values were also recorded on admission and 12 hours after delivery. All patients’ data including demographic parameters, intraoperative blood loss, hemoglobin levels, episiotomy repair time, and postoperative complications were recorded.

The SPSS (Version 11.5; SPSS Inc., Chicago, IL) statistical software was used for analyzing patient data. Normal distribu-

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Table 1. — Demographic and clinical characteristics of the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 20)</th>
<th>Study group (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.7 ± 3.9</td>
<td>21.1 ± 3.1</td>
<td>0.153</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1 (1-2)</td>
<td>1 (1-4)</td>
<td>0.463</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.300</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>278 (261-285)</td>
<td>276 (260-287)</td>
<td>0.849</td>
</tr>
<tr>
<td>Baseline hemoglobin (g/dl)</td>
<td>12.3 ± 1.05</td>
<td>12.6 ± 1.2</td>
<td>0.528</td>
</tr>
<tr>
<td>Postpartum hemoglobin (g/dl)</td>
<td>10.7 ± 1.2</td>
<td>11.6 ± 1.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Weight of sponge (g)</td>
<td>56.2 ± 39.0</td>
<td>31.1 ± 35.01</td>
<td>0.039</td>
</tr>
</tbody>
</table>

of the collected data was verified using the Kolmogorov-Smirnov test. When the Kolmogorov-Smirnov normality test revealed normal distribution, the independent sample t test for comparing the differences between two groups was used. When the test failed, the Mann-Whitney U test was used to compare the differences between the two groups. The level of significance was set at $p < 0.05$.

Results

In Table 1 the authors report demographic characteristics of the patients enrolled. Both groups were similar in terms of maternal age, parity, body mass index, and gestational age.

Baseline hemoglobin values measured on the admission showed no significant differences between the groups. The operative time for episiotomy repair for the two groups was also statistically insignificant. The weights of the sponges were heavier in the control group ($56.2 \pm 39.0$ g vs $31.1 \pm 35.01$ g). Hemoglobin on the first postpartum day was significantly higher in the ABS group ($p = 0.04$). Hemoglobin on the first postpartum day was $11.6 \pm 1.4$ g/dl for the ABS group and $10.7 \pm 1.2$ g/dl for the control group ($p = 0.04$) (Figure 1). No major immediate or delayed complications were observed in either group.

Discussion

Hemostatic agents have become increasingly employed across all surgical fields. Topical hemostatics are recommended for the management of low-volume bleeding rather than major hemorrhage. A plant extract, Ankaferd, was registered in Turkey as a “hemostatic agent” in 2007. ABS is a topical hemostatic agent and can be an alternative to manage external bleeding.

Several studies have been carried on to investigate the hemostatic capacity of Ankaferd in experimental traumatic bleeding models. It ensures a statistically-significant reduction in hepatic parenchymal bleeding [9]. Cipil et al. [10] evaluated in vivo hemostatic effect of ABS in rats pretreated with warfarin and found that ABS was indeed beneficial as a topical hemostatic agent.

ABS was also found to be safe and efficient in decreasing intraoperative bleeding when compared to the traditional hemostatic methods after cold-knife dissection tonsillectomy [6]. The same group also showed the efficacy of ABS in adults who suffered from epistaxis [4].

In this study, the hemostatic efficacy of ABS was investigated during episiotomy repair. To the authors’ knowledge, this is the first study evaluating the efficacy of ABS spray in terms of blood loss during episiotomy repair. In the study group, an application of 4 ml of ABS instead of isotonic saline solution lessened bleeding.

The present study revealed a positive effect of the topical application of ABS tested for bleeding reduction. The indications for topical ABS seem to increase in clinical settings. Nonetheless, like other hemostatic agents, ABS is expensive so that its use in episiotomy repair is controversial. Additional prospective studies with a larger number of patients are required to confirm these results.

References


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Type of delivery and self-reported postpartum symptoms among Iranian women

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¹Department of Midwifery, Babol University of Medical Sciences, Babol; ²Department of Midwifery, Fatemezahra Infertility and Reproductive Health Research Center, Babol University of Medical Sciences, Babol; ³Department of Midwifery, Mashhad University of Medical Sciences, Mashhad (Iran)

Summary

The aim of the present study was to examine the association between mode of delivery and self-reported postpartum among women eight weeks postpartum. A cross-sectional study was conducted on postpartum women with symptoms. A total of 300 individuals over 16 years (155 with normal vaginal delivery and 145 with elective cesarean section) from ten primary healthcare centers in an urban area of Amol, Mazandaran, Iran were selected using a clustering random sampling technique. A standard questionnaire named Edinburgh postnatal depression scale (EPDS) was used to assess depressive symptom. Most women (98.3%) reported at least one postpartum symptom at eight weeks postpartum. The most prevalent postpartum symptoms were excessive tiredness or fatigue (72.2%), pain (65.7%) and backache (61.3%). There was a decrease in percentage of occurrence of sexual problems (p = 0.009) with elective cesarean section at postpartum was founded. Compared with women having vaginal delivery, cesarean delivery women were more likely to report headaches (OR = 2.5; CI = 1.493, 4.289) and less to report sexual problems (OR = 0.594; CI = 0.362, 0.975) during postpartum. It would be useful to provide a defined standard for postpartum care and apply regular postpartum visits in primary health care centers, hospital, and home visits and restricting mediolateral episiotomy.

Key words: Postpartum; Vaginal delivery; Cesarean section; Birth; Depression.

Introduction

In Iran, over 1 million women give birth each year [1]. Iranian women receive normal prenatal care at primary health care centers (PHCs) [2]. More than 95% of births take place in the hospital (1) and in the 24 hours after delivery, mothers go home [3]. The quality of care in the postpartum ward of the hospital is weak [4] and there is no postpartum follow-up by discharge programs (3). The majority of mothers and clinicians do not concern the timing and content of antenatal care visits and postpartum care [5]. The mother’s postpartum care is very essential in women’s life and cannot be ignored. Women with cesarean child births have to be extra careful [6].

In Iran, the elective cesarean birth rate has risen each year and almost doubled during a five-year period [7] and 47% of deliveries are by cesarean section [8]. About 60% of women prefer to have cesarean to avoid labor pain or to determine the exact time of elective cesarean birth, thus we examined the association between mode of delivery (elective cesarean section and vaginal delivery) and self-reported postpartum symptoms among women at eight weeks postpartum.

Material and Methods

A cross sectional study was conducted on women attending urban PHC for a vaccination program of their baby eight weeks after delivery. A clustering random sampling method was used to select 300 women over 16 years old (155 with normal vaginal delivery and 145 with elective cesarean section) from ten PHCs in an urban area in Amol, Mazandaran, Iran. The ethics committee of Mashhad University of Medical Sciences and Mazandaran University of Medical Sciences approved the study. Informed consent was obtained from all women in the study.

Inclusion criteria were: being at least 16 years of age and having a full term singleton baby. Exclusion criteria: being under 16 years of age, having a multiple birth or preterm delivery, having psychological and pregnancy problems, serious medical disease, and emergency cesarean, and drug intake. Also, those with medical conditions such as low back pain, chronic constipation, urination problems before pregnancy, and history of depressive symptoms during pregnancy (also before and after pregnancy) were excluded from the study.

Mode of delivery was categorized to two categories: vaginal delivery and elective cesarean section. Vaginal delivery was defined as non-instrumental vaginal delivery and type of cesarean section included only elective cesarean. Women who had cesarean emergency after onset of labor and cesarean emergency with no labor (prelabor emergency) were excluded. Since at the time of the study, the Iranian version of questionnaires were not available, the questions on mother’s postpartum experiences were adapted from a questionnaire used by Brown et al. [9]. The reliability of the questionnaire was approved by Cronbach’s and it was 0.81. Moreover, its validity was determined by a panel of expert opinions. We assessed the prevalence of 12 common postpartum physical symptoms experienced and deprecative symptoms by women at eight weeks postpartum using the questionnaire.

In this study the Edinburg Postnatal Depression Scale (EPDS) was used; its validity and reliability have been assessed by Montazeri et al. and they showed that the EPDS is both reliable and valid in postnatal subjects in Iran [10].

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A socio-demographic questionnaire was used to obtain information regarding age, education, household income, insurance status, and occupational status. Other factors that were assessed included obstetric and delivery characteristics (e.g., parity, mode of delivery, perineal trauma) and psychosocial factors (e.g., unplanned pregnancy, and social support from partner or family).

**Statistical analysis**

Descriptive statistics were used to describe baseline socio-demographic, obstetric, and delivery, and social support variables. Differences in associations between groups were done using chi-square analysis. Multiple logistic regressions were used to determine the relationship between postpartum symptoms and mode of delivery. The odds ratio (OR) was presented used to determine the relationship between postpartum symptoms and mode of delivery. The odds ratio (OR) was presented together with the 95% CI. Adjustments were made for independent variables which included age, income, pregnancy planned, parity, occupation, and education. All analyses were employed using a two-tailed hypothesis with significance set at a p value of ≤ 0.05.

**Results**

Of the 348 women who attended PHCs, eight (2.3%) did not like participating in the research and 40 (11.5%) were excluded from analysis because of exclusion criteria; thus a total of 300 women were assessed and their characteristics are given in Table 1.

The mean age of the sample was 25.2 ± 4.7 years (range 17-40 years). More than 85% of subjects had an education of elementary level or lower. Only 23 (7.7%) were employed in the year prior to the birth. The mean parity of women was 1.4 ± 0.6 deliveries. Approximately 58% of women were primiparous and 95.7% were breast feeding at study entry. The mean number of antenatal visits for the last pregnancy was 9.5. Almost all the women (97.3%) had familial social support.

Table 2 shows the prevalence of postpartum symptoms and depression according to mode of delivery. The most prevalent postpartum symptoms were excessive tiredness or fatigue (72.2%), pain (65.7%), backache (61.3%), sore or cracked nipple (44.7%), and sexual problems (39.3%) at eight weeks postpartum.

In terms of women's depression, 14.0% of women experienced depression at eight weeks postpartum. An increase in percentage of occurrence of bad headaches (0.0001) and more cough or colds than controls (0.036) with elective cesarean section was observed, while, there was a decrease in percentage of occurrence of sexual problems (p = 0.009) with elective cesarean section at postpartum.

Table 3 illustrates the estimated OR (with 95% CI) and adjusted OR for risk of postpartum symptoms and depression. Compared with women undergoing vaginal delivery, women having cesarean section were significantly more likely to report headaches (OR = 2.528; CI = 1.493, 4.289) at eight weeks postpartum. Women with cesarean delivery were less likely to report sexual problems than those having vaginal deliveries (OR = 0.594; CI = 0.362, 0.975). Compared with women having vaginal delivery, cesarean delivery women were more likely to report more cough or colds than controls during postpartum (OR = 1.912; CI = 1.037, 3.525), but this did not reach statistical significance with adjusted OR.

**Discussion**

The postpartum period is an exciting, dynamic time in a woman’s life but both mother and clinician have little knowledge regarding physical or psychological state of women.
postpartum recovery. Postpartum care is very essential in a woman’s life, but often this care is ignored. The results showed that almost all of the women (98.3%) in the study reported at least one postpartum symptom since the birth. The majority of women (69.0%) reported between two and five symptoms, and those results are consistent with many studies indicating that only a small percent of women reported an absence of physical postpartum symptoms and over 90% of women had at least one postpartum symptom [11-13].

In this study the most prevalent reported postpartum symptoms at eight weeks postpartum were: excessive tiredness or fatigue, pain (perineal pain, incision of cesarean), and backache. These results are in line with many studies indicating that these symptoms are the most common symptoms.

Chi-square analysis revealed that there were significant differences between mode of delivery in three experienced postpartum symptoms; bad headaches, more coughs or colds than controls, and sexual problems. Women undergoing cesarean birth reported more postpartum symptoms at eight weeks postpartum than their counterparts who had a vaginal delivery.

Also these women reported needing more help during the postpartum period (83.4% vs 74.5%). Various researchers found that assisted vaginal delivery increased postpartum symptoms [14-16]. In Iran, assisted vaginal delivery is not common and there is an increasing trend toward cesarean rather than use of vacuum devices or forceps. Since none of our subjects reported that they had an assisted vaginal delivery, such concerns are not applicable to our study population.

Table 2. — Postpartum symptoms at eight weeks according to delivery.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total n (%)</th>
<th>Vaginal delivery = 155 n (%)</th>
<th>Elective cesarean = 145 n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessively tired or fatigued</td>
<td>218 (72.7)</td>
<td>114 (73.5)</td>
<td>104 (71.7)</td>
<td>0.723</td>
</tr>
<tr>
<td>Backaches</td>
<td>184 (61.3)</td>
<td>88 (56.8)</td>
<td>96 (66.2)</td>
<td>0.094</td>
</tr>
<tr>
<td>Sore or cracked nipples</td>
<td>130 (44.7)</td>
<td>73 (47.1)</td>
<td>61 (42.1)</td>
<td>0.381</td>
</tr>
<tr>
<td>Pain*</td>
<td>104 (47.1)</td>
<td>61 (42.1)</td>
<td>93 (64.1)</td>
<td>0.590</td>
</tr>
<tr>
<td>Hemorrhoids</td>
<td>15 (5.0)</td>
<td>8 (5.2)</td>
<td>7 (4.8)</td>
<td>0.895</td>
</tr>
<tr>
<td>Bowl problems</td>
<td>72 (20.0)</td>
<td>39 (25.2)</td>
<td>33 (22.8)</td>
<td>0.626</td>
</tr>
<tr>
<td>Bad headaches</td>
<td>95 (31.7)</td>
<td>35 (22.6)</td>
<td>60 (41.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Bladder problems</td>
<td>30 (10.0)</td>
<td>16 (10.3)</td>
<td>14 (9.7)</td>
<td>0.847</td>
</tr>
<tr>
<td>Red or tender breasts or mastitis</td>
<td>81 (27.0)</td>
<td>49 (31.6)</td>
<td>32 (22.1)</td>
<td>0.063</td>
</tr>
<tr>
<td>More cough or colds than control</td>
<td>52 (17.3)</td>
<td>20 (12.9)</td>
<td>32 (22.1)</td>
<td>0.036</td>
</tr>
<tr>
<td>Sexual problems</td>
<td>118 (39.3)</td>
<td>72 (46.5)</td>
<td>46 (31.7)</td>
<td>0.009</td>
</tr>
<tr>
<td>Depressive symptoms (EPDS &gt;12)**</td>
<td>42 (14.0)</td>
<td>118 (11.7)</td>
<td>24 (16.6)</td>
<td>0.226</td>
</tr>
<tr>
<td>No postpartum symptoms</td>
<td>5 (1.7)</td>
<td>0 (0.0)</td>
<td>5 (3.4)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

* Mothers having vaginal delivery were asked questions of perineal pain and mothers who had, elective cesarean were asked questions of pain of cesarean incision.
** Edinburgh Postnatal Depression scale (EPDS) measures point prevalence of depressive symptoms.

Table 3. — Odds ratio and 95% confidence interval for risk of postpartum and depressive symptoms: elective cesarean vs vaginal delivery.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Unadjusted or (95% CI)</th>
<th>Adjusted or (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessively tired or fatigued</td>
<td>0.912 (0.549-1.516)</td>
<td>0.825 (0.482-1.413)</td>
</tr>
<tr>
<td>Backaches</td>
<td>1.942 (0.340-3.833)</td>
<td>1.292 (0.794-2.103)</td>
</tr>
<tr>
<td>Sore or cracked nipples</td>
<td>0.816 (0.517-1.287)</td>
<td>0.790 (0.492-1.268)</td>
</tr>
<tr>
<td>Pain</td>
<td>0.877 (0.544-1.413)</td>
<td>0.881 (0.534-1.453)</td>
</tr>
<tr>
<td>Hemorrhoids</td>
<td>0.932 (0.329-2.639)</td>
<td>0.963 (0.327-2.833)</td>
</tr>
<tr>
<td>Bowl problems</td>
<td>0.876 (0.515-1.491)</td>
<td>0.813 (0.468-1.412)</td>
</tr>
<tr>
<td>Bad headaches</td>
<td>2.420 (1.466-3.990)*</td>
<td>2.528 (1.493-4.288)*</td>
</tr>
<tr>
<td>Bladder problems</td>
<td>0.928 (0.436-1.977)</td>
<td>0.936 (0.421-2.078)</td>
</tr>
<tr>
<td>Red or tender breasts or mastitis</td>
<td>0.613 (0.365-1.029)</td>
<td>0.590 (0.344-1.013)</td>
</tr>
<tr>
<td>Breast problems</td>
<td>0.876 (0.437-1.758)</td>
<td>0.904 (0.457-1.807)</td>
</tr>
<tr>
<td>More cough or colds than controls</td>
<td>1.912 (1.037-3.525)*</td>
<td>1.817 (0.964-3.426)</td>
</tr>
<tr>
<td>Sexual problems</td>
<td>0.536 (0.334-0.858)**</td>
<td>0.594 (0.362-0.975)**</td>
</tr>
<tr>
<td>Depressive symptoms (EPDS &gt;12)</td>
<td>1.499 (0.776-2.895)</td>
<td>1.686 (0.850-3.344)</td>
</tr>
</tbody>
</table>

* p < 0.01, **p < 0.05.
† Adjusted for age, income, planned pregnancy, parity, occupation and education.

Our study showed that around 67% of women with vaginal delivery reported perineal pain at eight weeks postpartum. In terms of pain associated with a cesarean section around 64% of all subjects reported pain at the site of incision. Glazener suggested that sexual problems were associated with perineal pain [17] and Hartmann et al. reported that dyspareunia was more common among women with episiotomy [18]. Another study showed no difference in reports of dyspareunia between women with medline episiotomy and women who had perineal tearing [19], while Baksu et al. found common sexual problems in women with mediolateral episiotomy [20].

In this study, nearly all of our subjects (143 of 150) had vaginal delivery by mediolateral episiotomy or perineal trauma and only nine (5.9%) had an intact perineum. Our results also showed that women with vaginal delivery had more sexual problems than women with cesarean sections at eight weeks postpartum. Our findings with respect to similarity of experiences between women who underwent cesarean section and women delivered vaginally by mediolateral episiotomy [20]. While another study showed no difference in sexual problems between women who delivered vaginally and those who underwent cesarean section [21]. A possible explanation for the higher prevalence of reported sexual problems is use of mediolateral episiotomy. We should put emphasis on restricting mediolateral episiotomy, although certainly our data do not support elective cesarean section as a strong protective effect on sexual problems at eight weeks postpartum.

This study showed excessive tiredness or fatigue is common at eight weeks postpartum and not related to mode of delivery. Several studies showed that tiredness was the most common postpartum symptom and generally related to mode of delivery [11, 12].

Several studies showed that approximately 10% of women will experience depression in the immediate postpartum period [22, 23], while we found more than 10% of
occurrences of depression at eight weeks postpartum did not relate to mode of delivery. A study from Iran reported that cesarean section is a certain risk factor for depression during postpartum [24].

There are several limitations of the study. We used a cross-sectional design to determine a relationship between mode of delivery and symptoms reported at eight weeks postpartum, whereas in future studies the use of longitudinal data should provide stronger evidence of this relationship. Moreover, this study is a self-reported outcome with sample restriction. However there was no issue of recall bias answering questions about postpartum symptoms at eight weeks as women were answering questions regarding their current postpartum symptoms.

In conclusion, this study showed a very high prevalence of postpartum syndrome at eight weeks postpartum. A possible explanation for the higher prevalence of postpartum symptoms is that the role of PHCs in postnatal care is unclear as is the use of mediolateral episiotomy. However PHCs are the only place that offer infant vaccination, and most women do visit PHCs at least to vaccinate their infants. Also PHCs provide prenatal care and family planning programs [10, 25]. Providing a defined standard for postpartum care and applying regular postpartum visits between three and eight weeks after delivery in PHCs and the hospital would be useful to reduce postpartum symptoms increase quality of life. Emphasis should be placed on counseling women during the prenatal visit regarding mode of delivery, postpartum services and related problems in terms of postpartum symptoms, contact with women who missed their postpartum appointment by phone and home visits and restricting mediolateral episiotomy.

Acknowledgments

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References


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Comparison of HbA1c levels in obese and non-obese polycystic ovarian patients

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Summary

Aim: To compare obese and non-obese polycystic ovary syndrome (PCOS) patients with respect to lipid profile, hormone profiles, and hemoglobin A1c (HbA1c) values indicating chronic hyperglycemia. Materials and Methods: Thirty PCOS patients with a body mass index (BMI) > 25 and 35 non-obese PCOS patients with BMI < 25 were compared with regard to basal luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), estradiol (E2), fasting blood sugar (FBS), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TCOL), triglyceride (TG), and HbA1c values. Results: HDL value (p = 0.005) was significantly higher in non-obese group while TG (p = 0.001) was higher in the obese group. No significant difference was found between other values. Conclusion: Lipid metabolism impairment seems to be more marked in obese PCOS patients. Moreover, it is obvious that insulin resistance is higher in obese group. The absent difference between obese and non-obese groups in terms of HbA1c values suggests that insulin resistance occurring in the obese group may also be important in the non-obese group. In this context, cardiovascular risks may increase in non-obese PCOS patients.

Key words: Polycystic ovary syndrome; Obese; HbA1c; Lipid metabolism; Insulin resistance.

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulation and affects about 5%-10% of women in their reproductive age. Its clinical symptoms comprise acne, hirsutism, hyperandrogenemia, and endocrinological effects of high levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), estradiol (E2), fasting blood sugar (FBS), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TCOL), triglyceride (TG), and HbA1c values. Results: HDL value (p = 0.005) was significantly higher in non-obese group while TG (p = 0.001) was higher in the obese group. No significant difference was found between other values. Conclusion: Lipid metabolism impairment seems to be more marked in obese PCOS patients. Moreover, it is obvious that insulin resistance is higher in obese group. The absent difference between obese and non-obese groups in terms of HbA1c values suggests that insulin resistance occurring in the obese group may also be important in the non-obese group. In this context, cardiovascular risks may increase in non-obese PCOS patients.

Materials and Methods

Overall, 65 PCOS patients referring to the gynecology outpatient clinic of this hospital between January 2009 and December 2011 were included in the present study. Detailed menstruation histories of all patients were elicited. Their ages were recorded. Height and weight were measured and the presence of hirsutism was detected with detailed examination. PCOS diagnosis was made according to revised Rotterdam criteria [9]. If they met at least two of following criteria, PCOS diagnosis was made:

1. Oligo-anovulation (less than six menstruations each year)
2. Clinical and biochemical symptoms of androgen excess including hirsutism (Ferriman-Gallwey score over 8, severe acne, and total testosterone levels over 0.8 ng/dl)
3. Ultrasonographic PCOS appearance [10].

Body mass index (BMI) of the patients included in the study was calculated by dividing their weight by meter square of their height. Accordingly, those with BMI over 25 were considered obese and those with BMI below 25 non-obese. There were 35 non-obese PCOS patients with BMI under 25 and 30 obese PCOS patients with BMI at or over 25. Patients with systemic disease other than PCOS, hypertension, diabetes, additional endocrinologic disease, and other gynecological pathologies were excluded from the study. Approval was obtained from the ethics committee and the study was carried out in accordance with the rules of Helsinki Declaration. Blood samples were drawn from all patients for the measurement of fasting follicle stimulating hormone (FSH), LH, estradiol (E2) and prolactin (PRL) hormone. In the determination of these parameters, immunoenzymatic method was used. In addition, fasting blood sugar (FBS), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TCOL), and triglyceride (TG) were examined by enzymatic colorimetric method. HbA1c analysis was made with high-performance liquid chromatography (HPLC) method. Data were transferred to computer with SPSS program and recorded as mean ± SD. In the comparison of the data, independent sample test was used.

Results

According to comparative results as shown in Table 1, no significant difference was found between the two groups in terms of age (p = 0.28), FBS (p = 0.16), LDL (p = 0.272), TCOL (p = 0.637), FSH (p = 0.398), LH (p = 0.297), PRL (p = 0.378), E2 (p = 0.591), and HbA1c
higher in the non-obese group. In the study carried out by et al. [4, 11]. On the other hand, no significant difference was observed between groups with regards to E2 and PRL levels. LDL and TCOL levels were not found to be significantly different between two groups while TG was significantly higher in the obese group and HDL was significantly higher in the non-obese group. According to these results, it may be stated that lipid metabolism disorders occur at a higher rate in obese PCOS patients. At this point, obese PCOS patients seem to be protected from future cardiovascular risks. These results stress the importance of weight-reducing diets in obese PCOS patients in order to decrease future cardiovascular risks [1].

There was no significant difference between two groups in terms of FBS values. When the authors considered HbA1c values, which is important as it shows blood glucose control within last three months, no significant difference was present between obese and non-obese groups. However, the value of 5.9 observed in the obese group, can be considered in the upper limit of normal. As it is known, HbA1c is an important value as it reflects mean blood glucose levels in the last two to three months. It shows chronic hyperglycemia and gives information not only on FBS levels but also on mean satiation blood sugar levels [8]. In PCOS, the relation between insulin resistance and metabolic syndrome is evident [3]. It is suggested that the most common cause of abnormalities in PCOS is insulin resistance [12]. Therefore, these patients run the risk of a higher rate of type II diabetes and have a four-fold higher cardiovascular disease risk [13]. This insulin resistance in PCOS has mostly been linked to obesity [4].

In the present study, no significant difference was found between obese and non-obese groups in terms of HbA1c values. These results is important since higher insulin resistance is expected in obese group. In previous studies [7], although high HbA1c value is weak diagnostic marker for diabetes, high HbA1c values in PCOS were linked to increased BMI and lipid profiles and indicates a possible increased risk of cardiovascular disease in PCOS patients. The present study demonstrated that HbA1c values in non-obese PCOS patients was similar to those in obese PCOS patients, which suggests that non-obese PCOS patients may also have higher risk of cardiovascular disease. Although it has low sensitivity for the diagnosis of diabetes mellitus [7], HbA1c levels is considered a gold standard in some studies for some cardiovascular events and mortality [14].

In addition, in some population-based studies, a close relation between BMI, lipid profiles, and HbA1c indicate that HbA1c may be utilized as an inflammatory marker in PCOS [15]. In conclusion, although the association of HbA1c and BMI is regarded important in obese PCOS patients, similar values in both groups obtained in our study, should alert us to the fact that cardiovascular risks may also be present in non-obese PCOS patients, suggesting that recommendations of life style changes and diet should be made for non-obese PCOS patients as well. Thus, HbA1c values should be measured in non-obese patients with the suspicion of PCOS and their probable cardiovascular risks should be kept in mind.

**Discussion**

Clinical and biochemical comparisons were made between obese and non-obese PCOS in various studies. In some of these studies, severe ovulatory dysfunction and higher serum total testosterone levels were found in the obese group [11]. In the present study, patients were standardized in terms of clinical examination findings (ovulation, acne, and hirsutism) and were compared with respect to biochemical and hormone laboratory parameters. In addition, HbA1c levels, which yield information on long-term blood glucose levels, were also compared.

Although mean LH values were found to be lower in the obese group, the difference between the two groups was not significant. Likewise, FSH level was also lower in the obese group without statistical significance. These results show that increase in LH/FSH ratio, which is the indicator of anovulation, is more marked in the obese group, which is in keeping with the results in the literature [4, 11]. On the other hand, no significant difference was observed between groups with regards to E2 and PRL levels. LDL and TCOL levels were not found to be different between two groups while TG was significantly higher in the obese group and HDL was significantly higher in the non-obese group. In the study carried out by El-Mazny et al. [1], all lipid metabolism products were found to be higher in insulin-resistant group whereas in the present study, TG was higher in the obese group with HDL being higher in the non-obese group. According to these results, it may be stated that lipid metabolism disorders

<table>
<thead>
<tr>
<th>Table 1. — Summary of results.</th>
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<tr>
<td><strong>BMI</strong></td>
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<tr>
<td>Age</td>
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<tr>
<td>&lt; 25</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>FBS</td>
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<tr>
<td>96 ± 9.1</td>
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<tr>
<td>HDL</td>
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<tr>
<td>41 ± 8.4</td>
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<tr>
<td>LDL</td>
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<tr>
<td>108 ± 34.8</td>
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<tr>
<td>TCOL</td>
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<tr>
<td>179 ± 31.7</td>
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<tr>
<td>TG</td>
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<tr>
<td>145.7 ± 68.9</td>
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<tr>
<td>FSH</td>
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<td>6.02 ± 1.3</td>
</tr>
<tr>
<td>LH</td>
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<tr>
<td>9.6 ± 6.9</td>
</tr>
<tr>
<td>E2</td>
</tr>
<tr>
<td>58 ± 36</td>
</tr>
<tr>
<td>PRL</td>
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<tr>
<td>14.1 ± 9.5</td>
</tr>
<tr>
<td>HbA1c</td>
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<td>5.91 ± 1.07</td>
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(p = 0.243) levels. However, HDL was significantly higher in (p = 0.005) non-obese group while TG (p = 0.001) was higher in the obese group (Table 1).


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Administration of lopinavir/ritonavir association during rat pregnancy: maternal and fetal effects

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Summary

Purpose: To evaluate the effects of the association of lopinavir and ritonavir administered during the whole period of rat pregnancy. Methods: 62 Wistar rats of the EPM-1 variant weighing about 200 g were randomly divided into five groups: two controls (Ctrl1 = stress control, n = 10; and Ctrl2 = drug vehicle control, n = 10) and three experimental ones which were treated with an oral solution of lopinavir/ritonavir (Exp1 = 12.8/3.2 mg/kg b.w., n = 14; Exp2 = 38.4/9.6 mg/kg b.w., n = 14; Exp3 = 115.2/28.8 mg/kg b.w., n = 14) from ‘day 0’ up to the 20th day of pregnancy. Maternal body weight was recorded at the start of the experiment and on the 7th, 14th and 20th day thereafter. At term (20th day), upon laparotomy and hysterotomy, the rats were anesthetized and the amount of implantations, reabsorptions, living fetuses, placentae and intrauterine deaths were recorded. The collected fetuses and placentae were weighed and the concepts were examined under a stereoscope microscope for external malformations.

Results: An apparent dose-unrelated lethal effect of the antiviral association on the pregnant rats was observed; notwithstanding, the body weight gain of the surviving rats had no changes, independent of the considered group. It was noted that the quantitative and qualitative intrauterine content of living term rats was indistinguishable from that of the controls. Conclusion: There was some degree of deleterious effects of the administration of the lopinavir/ritonavir association on pregnant rats; such effects eventually led to maternal death. However, neither the surviving rats showed toxicity nor did their concepts present any detectable change which could be related to the drug association.

Key words: Rat; Pregnancy; Lopinavir; Ritonavir.

Introduction

After its initial description in two small groups of previously healthy homosexual men [1, 2], the acquired immunodeficiency syndrome, so-called AIDS [3], evolved to constitute a pandemic.

Currently, in addition to the 20 million deaths registered till now, it is estimated that more than 40 million people worldwide are infected by the human immunodeficiency virus (HIV). The incidence distribution among men and women [4], mainly during the reproductive years is similar. It is thought that infected women are responsible for 20% of intrauterine transmissions [5, 6].

The most effective approach to the prophylaxis of such a vertical transmission is reduction of the viral load of pregnant women down to as low as 1,000 viral particle copies per milliliter [7].

The use of zidovudine during pregnancy and delivery has reduced HIV infection in one-third of the concepts [5]. Accordingly, the US Public Health Service has recommended that HIV-infected women continue with the same treatment protocol during gestation adopted in the pre-conception period [8].

Among the several treatment protocols, the highly active antiretroviral therapy (HAART) usually includes one nucleoside analog (DNA chain terminator), one protease inhibitor and either a second nucleoside analog (“nuke”) or a non-nucleoside reverse transcription inhibitor [9]. Though the association of antiretroviral drugs has been shown to be largely safe for the concept [10], it is supposed that association of multiple drugs may introduce changes in their pharmacokinetics [11] and bring about unpredictable results for maternal and/or fetal compartments.

It is well established that HIV antiretroviral drugs, particularly protease inhibitors (e.g., ritonavir and lopinavir) frequently elicit a metabolic syndrome that may include hyperlipidemia, lipodystrophy and insulin resistance. Cao et al. [12] in studies in vitro have demonstrated that most protease inhibitors not only induce the accumulation of intracellular free cholesterol and lipids, activating the urokinase-type plasminogen activator (UPR) in hepatocytes and macrophages but also increase the release of inflammatory cytokines promoting foam cell formation in macrophages [13, 14].

Pistell et al. [15] showed that lopinavir/ritonavir administration in mice caused significant metabolic derangement, including changes in body weight and fat mass as well as dose-dependent patterns of hyperlipidemia, hypoadiponectinemia, hypoleptinemia, and hyperinsulinemia.
Figure 1. — Percentual profiles of body weight gain among pregnant rats as a function of the starting weight (at the zero day of pregnancy). Groups comprised: Ctr 1 = intact, stress control, n = 10; Ctr 2 = drug vehicle (propyleneglycol) control, n = 10; Exp 1, 2 and 3 = treated with lopinavir/ritonavir association, 12.8/3.2 mg/kg (n = 14), 38.4/9.6 mg/kg (n = 14) and 115.2/28.8 mg/kg b.w. (n = 14) respectively, from day 0 up to the 20th day of pregnancy. No significant differences were observed.

Since preliminary results in our laboratory [16] have indicated some degree of toxicity of the lopinavir/ritonavir association on the rat pregnancy, this point has been specifically studied in this paper.

Materials and Methods

Wistar female rats (Rattus norvegicus albinus) of the EPM-1 variant, provided by the Center for the Development of Experimental Models (CEDEME) of Sao Paulo Federal University – School of Medicine (UNIFESP-EPM), weighing approximately 200 g, were used throughout the experiment. The experiment was approved by the local Animal Care Committee (Report no. 1397/04), in accordance with the guidelines which comply with those of the Canadian Council on Animal Care [17].

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

After a 7-day period of adaptation, the animals were mated in the proportion of one male to three females for two hours. The immediate 24-hour period after mating was taken as day 0 of pregnancy if spermatozooids were detected in vaginal smears [18]. Sixty-two pregnant rats were randomly divided into five animal groups, as follows: Ctr 1 (n = 10) was represented by rats which received no drugs, that is, the stress control; Ctr 2 (n = 10) was a group daily treated with 0.5 ml of propyleneglycol by oral route (drug vehicle control); Exp 1 (n = 14) was a group treated with the association of lopinavir/ritonavir (Kaletra, Abbott Laboratories, IL, USA) by oral route corresponding to a daily dose of 12.8 mg/kg lopinavir/3.2 mg/kg of ritonavir; Exp 2 (n = 14) was a group treated daily with 34.4 and 9.6 mg/kg of lopinavir and ritonavir, respectively; Exp 3 (n = 14) was a group treated daily with 115.2 and 28.8 mg/kg of lopinavir and ritonavir, respectively. Vehicle and drugs were administered by gavage, once daily, in a final volume of 0.5 ml, starting at day ‘0’ and extending until the term of pregnancy.

Body weights were recorded for all animals on day 0, and the 7th, 14th and 20th day of pregnancy and expressed as percentages of body weight gain.

At term (20th day), the animals were weighed and anesthetized with a mixture of xylazine (20 mg/kg) and ketamine (100 mg/kg) by the intraperitoneal route. Upon wide open laparotomy and hysterotomy, the following parameters were recorded: fetal and placental weights, number of implantations, reabsorptions, living and dead fetuses. The fetuses were closely examined under a stereoscope microscope for gross external malformations (limb shortening, spina bifida, cleft lip, cleft palate and hypospadias).

Whenever appropriate the data were expressed as mean ± SEM. The results were submitted to ANOVA analysis and analyzed further by the Kruskal-Wallis’ multiple comparison test. Contingency tables and chi-square tests were used to analyze the death rates; the significance level was set at 5%.

Results and Discussion

At advanced stages of HIV disease, patients used to show constitutional symptoms and severe cachexia. However, upon the development of HAART protocols [19], metabolic abnormalities (hyperlipidemia and insulin resistance) and morphological changes (central fat accumulation and peripheral fat atrophy) have been reported in HIV-1 patients mostly among those receiving therapies containing HIV-1 protease inhibitors, particularly ritonavir [20]. The so-called lipohypertrophic syndrome [21] includes clinical features as increased abdominal girth [22], fat accumulation, breast hypertrophy and buffalo hump [23].

In our study, pregnant rats treated with lopinavir/ritonavir showed no significant alteration in body weight gain, nor did the controls or experimental groups (Exp 1-3) (Table 1). This finding was presumably due to the fact that rats lack some of the multiple factors involved in the pathogenetic mechanisms proposed for humans, which
include the interference of several regulatory proteins such as sterol regulatory enhancer binding protein-1, the proteasome, mitochondrial DNA polymerase gamma and GLUT-4 [24]. Otherwise, the short time of exposition to the drugs (3 weeks) could have played some role in this result [25].

We observed a lethal effect of the drug treatment, that is, 4/14 deaths were recorded in the Exp 1 group (treated with a dose of lopinavir/ritonavir proportionally similar to that used in humans), and a two-fold higher figure was observed in the Exp 1 and 2 groups (treated with 3- and 9-fold higher doses of the drugs) (Table 1).

This finding addresses two relevant issues. First, no clear-cut dose-dependent effect can be inferred for this result. On the other hand, it may instead be related to the individual sensitivity of the animals to the drugs. Such individual variation could be linked to the functional levels of P glycoprotein (P-gp) [26], a membrane efflux pump pertaining to the superfamily of ATP binding cassette proteins [27]. As is known, this protein is encoded by the ‘multidrug resistance’ genes Mdr 1a and Mdr 1b [28] and expressed in tissues involved with drug absorption, metabolism and excretion [29]. P-gp limits penetration of potentially harmful or therapeutic hydrophobic compounds, thus providing protection of an organism against potentially toxic compounds of the environment. Accordingly, a placental P-gp may play an important role in the protection of the developing fetus [30]. Other barriers in which P-gp is importantly involved are the blood-brain, the blood-nerve, the blood-testis and the intestinal barrier [31]. Predictably, the absence of P-gp may lead to adverse effects when an organism is exposed to drugs such as antineoplastic agents, cardiac glycosides, beta-blockers, calcium channel blockers and HIV-protease inhibitors [32].

It is conceivable that the maternal deaths observed herein were due to some degree of individual variation of the intestinal content of P-gp, leading to defective drug protection [30]. Such ‘drug inward leakage’ at the intestinal, absorptive level, could have resulted in blockade of the metabolic degradation of lopinavir as a consequence of CYP3A isoenzyme inhibition caused by ritonavir [33-35]. This drug accumulation could be accounted for by the maternal deaths observed in our groups Exp 2 and 3.

Our results are supported in part by previous data obtained in our laboratory with ritonavir alone [36]. This drug was administered (60 and 180 mg/kg b.w.) during the entire period of rat pregnancy and caused 2/10 and 4/10 deaths, respectively. In the present paper, much lower ritonavir doses in association with lopinavir were able to cause a higher lethal effect, most presumably due to the association-induced, altered drug pharmacokinetics [11].

Regarding the fetal compartment, we may infer that the placental expression of P-gp [32] was sufficient as to properly hinder the drug transfer to the uterine space. In fact, no alterations were observed regarding the numbers of implantations and fetuses, and the fetal and placental weights between the control and the treated groups of animals. Similarly, no reabsorptions, intrauterine deaths or fetal malformations were observed.

To conclude, though maternal deaths have been recorded in the animal groups treated with the two highest doses of lopinavir/ritonavir, no definite dose-related effects could be established. On the other hand, the fetuses from surviving rats showed no detectable alterations, suggesting the efficacious operation of protective drug systems at the placental level.

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Surgical repair of a complicated urethro-vaginal fistula: case report and review of the literature

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Introduction

Urethro-vaginal fistulae (UVF) are uncommon in the developed world. They usually occur as an infrequent complication of a variety of gynecological surgical procedures. Anti-incontinence procedures, urethral diverticulectomy, and placing of bladder neck (autologous) slings are considered to be common surgeries that may lead to UVF. Surgeries for anterior vaginal wall prolapse, radiation therapy, and operative vaginal delivery can cause UVF due to tissue ischemia, problems related to healing, or radiation necrosis. Congenital UVF are considered to be extremely rare.

The aim of this study was to present the management of an interesting case of a complicated UVF diagnosed after gynaecological surgery. The authors report all surgical steps that were followed with an attempt to analyze available data from current literature as well.

Case Report

The patient, a 61-year-old gravida-2, para-2, post-menopausal woman, underwent anterior and posterior vaginal wall repair in a district general hospital due to cystocele and stress urinary incontinence (SUI). She had undergone anterior and posterior vaginal wall repair due to cystocele and stress urinary incontinence (SUI). Transvaginal repair was performed 20 weeks after primary surgery. However, a second transvaginal reconstructive surgery using Martius-flap originating from the bulbocavernous muscle was necessary due to persistent urine leakage in the vagina. Thirty-two months after successful urethro-vaginal treatment, the patient self-referred for persistent SUI. Burch colposuspension was performed and at 16 months follow-up the patient remains continent.

Discussion:
Surgical repair of complicated UVF seems to be more successful with Martius flap interposition than with no interposition.

Key words: Urethro-vaginal fistula; Martius flap; Burch colposuspension; Stress urinary incontinence.

Summary

Background: Urethro-vaginal fistulae (UVF) occur usually as infrequent complications of a variety of gynecological surgical procedures. The aim of this study was to present an interesting case of a complicated UVF diagnosed after gynaecological surgery. Case: A 61-year-old gravida-2, para-2, post-menopausal woman was referred with a complaint of urine loss through the vagina. She had undergone anterior and posterior vaginal wall repair due to cystocele and stress urinary incontinence (SUI). Transvaginal repair was performed 20 weeks after primary surgery. However, a second transvaginal reconstructive surgery using Martius-flap originating from the bulbocavernous muscle was necessary due to persistent urine leakage in the vagina. Thirty-two months after successful urethro-vaginal treatment, the patient self-referred for persistent SUI. Burch colposuspension was performed and at 16 months follow-up the patient remains continent. Discussion: Surgical repair of complicated UVF seems to be more successful with Martius flap interposition than with no interposition.

Case Reports
Discussion

The ideal timing and appropriate surgical procedure for the repair of UVF are still debated [1]. Good quality healthy vaginal tissue is considered to be one of the main factors that favor successful outcome, so early repair after injury is not most likely the best approach. For cases in which tissue is otherwise healthy, early vaginal repair within two to three weeks of injury is possible without increased morbidity or failure rates [2]. Other studies suggest a wait of 8-12 weeks before repair [3]. In the present case the patient was referred 12 weeks after injury, so early repair was not an option.

Preoperative cystoscopy should be used to evaluate the anatomic relationship of the fistula. Also urodynamic study is always preoperatively necessary as there is a high incidence of abnormal lower urinary tract function in patients with urogenital fistulae [4]. Patients with UVF have high incidence of both genuine stress incontinence and detrusor instability. Many of these abnormalities appear to resolve after successful repair of the fistula, although detrusor instability may persist and require further treatment in some women. On the other hand, surgical repair of urogenital fistulae may lead to SUI. These data are relevant to the counseling of patients before repair and may be of medico-legal significance. In the present case, urodynamic study was performed before the first transvaginal reconstructive surgery, as well as before Burch colposuspension. Both studies confirmed the diagnosis of urodynamic stress incontinence.

Transvaginal repair is the preferred method to avoid the morbidity of laparotomy and to provide a more rapid recovery of the patient. On the other hand, suprapubic or combined surgical procedures should be reserved for situations in which access to the fistula is limited. Generally, a vaginal approach is recommended at the primary reconstruction of UVF, whereas a combined suprapubic and vaginal approach is recommended in vesicovaginal fistulae as well as in recurrent UVF fistulae [5].

Martius interposition of the omentum, muscle, peritoneum or labial fat has better results in recurrent or complicated fistulae. These grafts are appropriate to eliminate tissue tension, to fill a defect in the vagina, and to establish neovascularity. In the present case, the use of a Martius flap in the second surgery led to a successful result. The increased degree of fibrosis in the suburethral region after the previous transvaginal surgical procedures led to Burch colposuspension instead of placing a suburethral tension-free tape for the final management of urodynamic stress incontinence, in order to avoid postoperative erosion. This procedure was decided after identification of normal bladder neck mobility. Urethral drainage for at least 21 days seems to be necessary and the delay before resuming sexual activity must be individualized for each case.

Conclusions

The principles of fistula surgery are well-established: visualization of the tract, a tension-free watertight closure, assurance of adequate vascular supply to the repair, and appropriate bladder drainage.

References


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Misoprostol for labor induction in the second trimester in a woman with previous three cesarean deliveries and an intrauterine death of an anencephaly

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Summary
Termination of pregnancy in the second trimester for an intrauterine death of a fetus with anencephaly in a woman with previous three cesarean sections is a difficult clinical dilemma. A 34-year-old, gravida 4, para 3 woman was admitted at 20 weeks gestation for termination of pregnancy due to intrauterine death of a fetus with anencephaly. She had had three previous cesarean sections. She received two doses of 200 mcg misoprostol tablets vaginally 12 hours apart. Then two doses of 400 mcg misoprostol tablets were given vaginally 12 hours apart. There were no uterine contractions or cervical changes. Finally, she received five doses of 400 mcg misoprostol tablets vaginally every eight hours. The patient responded after the last dose and the fetus with the placenta aborted completely without complications. The estimated blood loss was 200 ml. Conclusion: Misoprostol can avoid hysterotomy for termination of pregnancy in the second trimester with history of previous three cesarean sections and an intrauterine death of a fetus with anencephaly.

Key words: Misoprostol; Anencephaly; Three cesarean deliveries.

Introduction
Misoprostol is a synthetic prostaglandin E₁ analogue readily absorbable sublingually, bucally, vaginally or rectally [1]. Clinical guidelines and recommendations exist for the use of misoprostol for second trimester termination of pregnancy in the unscarred uterus, but not for the scarred uterus [2]. There is increasing body of evidence to support the safe use of misoprostol in the second trimester termination of pregnancy in women with one previous cesarean section [3, 4]. However, there are very few published reports on the use misoprostol in women with previous three cesarean sections, but not with anencephaly. Medical termination of pregnancy due to anencephaly is difficult. The aim of this case report is to present the successful use of misoprostol for termination of pregnancy in the second trimester in a woman with previous three cesarean deliveries and an intrauterine death of a fetus with anencephaly.

Case report
A 34-year-old, gravida 4, para 3 woman was admitted at 20 weeks of gestation for termination of pregnancy due to the intrauterine death of a fetus with anencephaly. She had had three previous cesarean sections. On examination, she was in good medical condition. Vaginal examination revealed closed, thick, and a 3.5 cm long cervix. The options were discussed with the couple to either terminate the pregnancy by misoprostol or by hysterotomy. The couple decided to terminate the pregnancy by misoprostol after detailed counseling of the risks and benefits. She received two doses of 200 mcg misoprostol tablets vaginally 12 hours apart. Then she received two doses of 400 mcg misoprostol vaginally 12 hours apart. There were no uterine contractions or cervical changes. Finally, she received five doses of 400 mcg misoprostol vaginally every eight hours. The patient responded six hours after the fifth dose of 400 mcg misoprostol vaginally and the fetus with the placenta were aborted completely without complications. The estimated blood loss was 200 ml.

Discussion
Second trimester abortion comprises 10-15% of the 42 million abortions that occur worldwide each year [5]. With the pandemic increase in the rates of cesarean section for various reasons, it is more likely to encounter women for termination in the second trimester with history of previous cesarean section [6]. Based on accumulating evidence, the use of misoprostol for termination in the second trimester in women with one previous cesarean section is safe. The risk of rupture of the uterus with a prior cesarean delivery was 0.28% (95% CI, 0.08 - 1.0) compared to 0.04% (95% CI, 0.02 - 0.20) in women without cesarean delivery [7]. Therefore, the International Federation of Gynecologists and Obstetricians recommended the use of misoprostol for termination of pregnancy from 18 to 26 weeks gestation in women with previous cesarean section [8]. Second trimester termination of pregnancy for intrauterine death of a fetus with anencephaly in a woman with previous three cesarean sections is a clinically difficult situation. Hysterotomy may sometimes be necessary despite the fact that it is associated with high morbidity and even mortality [9]. On the other hand, there is insufficient evidence to recommend the use of misoprostol in women with previous multiple cesarean deliveries [10]. However, Fawzy and Abdel-Hady, in...
2010, reported the use of misoprostol in 31 women with second trimester abortion and three or more cesarean sections [11]. Misoprostol was inserted vaginally every six hours, a 200-μg tablet for the first 24 hours and two tablets thereafter, until regular uterine contractions were observed, or the products of conception were expelled. Women who did not abort within 48 hours of misoprostol received additional doses of misoprostol; or received a transcervical Foley catheter with extra-amniotic Pgf2α instillation 0.5 mg every two hours; or received an intravenous infusion of oxytocin. If abortion did not occur within 72 hours of the first misoprostol insertion, the treatment was considered to have failed completely and the option of hysterotomy was discussed with the patient. Vaginal abortion was achieved in 28 women (90.3%) and three women (9.3%) needed hysterotomy. When compared to a control group of 107 women with an unscarred uterus, the rate of severe hemorrhage and blood transfusion were similar in the two groups. In the current case, the longer time to achieve the termination may be due to the fact that the dead fetus had anencephaly. In addition, neither transcervical Foley catheter with extra-amniotic Pgf2α instillation nor intravenous oxytocin were used. Misoprostol avoided hysterotomy for termination of pregnancy in the second trimester in a woman with previous three cesarean deliveries and intrauterine fetal death of anencephaly.

References


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Repeated term pregnancies in a young patient with pelvic organ prolapse

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Summary

Pregnancy complicated with pelvic organ prolapse is a rare event; pregnancy in a patient with prolapse existing before the pregnancy is even less common. The authors report two consecutive term pregnancies in a young woman with pelvic organ prolapse. A 24-year-old pregnant woman, gravida 4, para 3, was admitted to the hospital at 38 2/7 weeks gestation with uterine contractions and severe cervical prolapse. She was conservatively treated during the antenatal period. On admission, an edematous and gangrenous totally prolapsed cervix was seen protruding outside the introitus and cesarean section was then performed. A healthy female infant with a birth weight of 2,920 g was delivered. On postpartum second day examination, uterine cervix was reduced. Stage 2 pelvic organ prolapse quantification system (POQ) was observed during the follow-up examination at sixth weeks postpartum. Conservative approach during pregnancy followed by cesarean section may be the appropriate management in these cases.

Key words: Prolapse; Pregnancy; Management; Preexisting.

Introduction

Pelvic organ prolapse during pregnancy is an extremely rare event with a reported incidence of one in 10,000 to 15,000 deliveries in the United States [1]. There were only five cases reported from 1968 to 2005; the authors described rare and extensive prolapses with elongation of the cervix during pregnancy [2]. Pregnancy in a patient with prolapse existing before the pregnancy is even less common with four cases reported from 1980 to 2010 [3-6] and four additional cases from 2010 to 2011 [7-10].

Pregnancy with uterine prolapse rarely extends to term carrying the risks of cervical desiccation and ulceration, urinary retention, cervical dystocia, preterm labor, spontaneous abortion, fetal demise, maternal sepsis, and death [11, 12]. Treatment options are limited with conservative methods during antenatal period. Management during labor varies considerably.

The authors report a case of two consecutive term pregnancies with preexisting pelvic organ prolapse and a review of the literature.

Case Report

A 24-year-old pregnant woman, gravida 4, para 3, was admitted to the tertiary care hospital at 38 2/7 weeks gestation with uterine contractions and severe uterine prolapse. The patient’s past medical and surgical history was unremarkable. Her two previous pregnancies resulted with preterm vaginal deliveries six and four years ago. The weights of newborns were 2,500 and 2,000 g respectively. She had no history of prolapse during these two prior pregnancies. However, she reported that the cervix had protruded outside the vaginal introitus after the second delivery. Her third pregnancy ended with spontaneous vaginal delivery of a term infant of 3,500 g three years ago. The cervix had been reduced manually at the sixth month of that pregnancy. The patient took antenatal care in another center during this pregnancy and stated that prolapse recurred at the seventh month. The patient was conservatively treated with manual reduction, bed rest, and followed on an outpatient basis. She told that she had felt the mass and seen that it was protruded in the lower vagina on the morning of the day she was admitted to the hospital. On admission, an edematous, incarcerated, and totally prolapsed cervix was seen protruding outside the introitus (Figure 1). Ultrasonographic examination showed a single, live fetus with an estimated fetal weight of 3,000 g. The cardiotocography revealed regular uterine contractions with normal fetal heart rate pattern. As the gangrenous and necrotic appearance of the cervix revealed that its circulation was compromised, in order to avoid potential risk of cervical laceration and dystocia, delivery by cesarean section was decided. A healthy female infant with a birth weight of 2,920 g was delivered with Apgar scores of 7 and 9 at the first and fifth minutes, respectively. The patient requested sterilization. Tubal sterilization was performed during cesarean section with the informed consent given by the patient. On postpartum second day examination, a reduced and healing uterine cervix was observed. The postoperative period was uncomplicated and the patient was discharged on postoperative day two. Stage 2 pelvic organ prolapse quantification system (POQ) was observed on the follow-up examination at sixth week postpartum.

Discussion

This report summarizes a case of pelvic organ prolapse while complicating two pregnancies of a young patient. Pelvic organ prolapse is considered to be a multifactorial condition in which both congenital and acquired factors are blamed. Congenital weakness in the fascial supports as well as multiparity, previous macrosomic births, operative delivery, and large uterine or ovarian tumors resulting in increased intra-abdominal pressure are among the most common risk factors [12]. Childbirth trauma is the first cause of most reported cases of uterine prolapse and pregnancy during the reproductive years. In addition, the
physiologic changes of pregnancy, in terms of cervical elongation and hypertrophy, may also be contributing factors for uterine prolapse [13].

During pregnancy, prolapse is usually first noted in the third trimester [14] and disappears after labor and delivery [13]. However, spontaneous resolution will usually occur by the end of the second trimester without further complications but persists or recurs after delivery in cases where prolapse existed before the onset of pregnancy [9, 12]. However, in the previous two reports, prolapse persisted during the entire pregnancy [3, 5], and in one case the prolapse was resolved at the 30th week of pregnancy [7]. In the presented case, the prolapse seemed to persist during pregnancy and increased gradually. The condition became extensive and was then manually reduced at the seventh month of pregnancy. Insertion of a pessary to protect the prolapsed cervix may be attempted although it may fall out a few days later [6]. Thus, treatment options are limited with conservative methods, such as genital hygiene and bed rest, in a slight Trendelenburg position during the antenatal period [12]. A laparoscopic approach of uterine suspension was also reported as an alternative solution where the conservative precautions failed [15].

Resistance to cervical dilatation, cervical dystocia due to edema, cervical laceration, and obstructive labor with an incidental risk of rupture of the lower uterine segment have been reported as intrapartum complications [13]. Although operative vaginal delivery by forceps application or Dührssen cervical incisions may be attempted, continued stretching of the lower segment to the point of uterine rupture due to cervical dystocia has been reported [1, 5]. Another complication may be primary postpartum bleeding due to uterine atony [16]. In order to avoid such complications, an elective cesarean section near term seems to be the safest mode of delivery in cases with edematous and elongated cervix [17]. In the presented case, as the gangrenous and necrotic appearance of the cervix revealed that its circulation was compromised, in order to avoid potential risk of dystocia and cervical laceration, delivery by cesarean section was decided.

Cesarean hysterectomy with suspension operation may be a radical approach especially for women who completed their family [5]. However, this patient was young and desired to have her uterus preserved. Furthermore, the prolapse could be reversible and hysteropexy operation would be unsuccessful when the uterus returned to pelvis minor within a few weeks after the delivery [16]. In case of pelvic organ prolapse, vaginal procedures are considered as the primary ones for pelvic reconstructive surgery.

In conclusion, the authors wanted to add this case to the limited ones in the literature to review this rare entity and to help the obstetricians to be familiar with management of these cases. Close follow-up with conservative precautions is recommended during the antenatal period. Cesarean section seems to be the safest way of delivery. However, management should be individualized according to the age of the patient, gestational week, severity of the condition, and patient’s preference.

References

Repeated term pregnancies in a young patient with pelvic organ prolapse


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Ultrasound diagnosis of recurring Jeune’s syndrome: a case report

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Summary

Jeune’s Syndrome or asphyxiating thoracic dystrophy (ATD) is a rare autosomal recessive skeletal dysplasia syndrome characterized by a small and narrow chest, short extremities, and often polydactyly associated with multiple organ manifestations. Its estimated incidence is one in 100,000 to 130,000 live births [1]. The diagnosis is based on clinical and radiological findings. Pathognomonic radiological findings at birth include bell-shaped thorax with short horizontal ribs, square-shaped iliac wings, and metaphyseal widening [1, 2]. This report demonstrates the importance of prenatal diagnosis of the syndrome and the ethical dilemmas that arose regarding poor prognosis and low quality of life of these patients. The authors are also attempting to highlight key points to antenatal sonographic diagnosis. The cases refer to the recurrence of the syndrome in consecutive pregnancies of a non-consanguineous couple.

Key words: Jeune’s Syndrome; Asphyxiating Thoracic Dystrophy; Ultrasound diagnosis of Jeune’s Syndrome.

Introduction

Jeune’s syndrome or asphyxiating thoracic dystrophy (ATD) is a rare autosomal recessive skeletal dysplasia clinically characterized by a small and narrow chest, shortness of all four extremities, and postaxial polydactyly in 14% of cases, associated with multiple organ anomalies. Its estimated incidence is one in 100,000 to 130,000 live births [1]. The diagnosis is based on clinical and radiological findings. Pathognomonic radiological findings at birth include bell-shaped thorax with short horizontal ribs, square-shaped iliac wings, and metaphyseal widening [1, 2]. This report demonstrates the importance of prenatal diagnosis of the syndrome and the ethical dilemmas that arose regarding poor prognosis and low quality of life of these patients. The authors are also attempting to highlight key points to antenatal sonographic diagnosis. The cases refer to the recurrence of the syndrome in consecutive pregnancies of a non-consanguineous couple.

Case Report

A 19-year-old woman was referred to this clinic for fetal evaluation. Detailed sonography at 24 weeks of gestation showed shortness of all long bones (below fifth centile) and a small narrow thorax. Measurements of all long bones were below the third centile and the chest was small with short ribs. Serial scans confirmed the findings and also showed polydactyly of the left hand. Amniocentesis was performed and a normal male karyotype and normal alpha-fetoprotein levels were diagnosed. The couple opted once again for pregnancy termination.

Discussion

The syndrome demonstrates a variety of clinical manifestations that vary from mild to lethal. The prominent problem is hypventilation caused by impaired chest expansion and subsequent pulmonary hypoplasia [3]. Pulmonary insufficiency or respiratory distress syndrome (RDS) is the main cause of death among patients before the second year of life with a percentage that reaches 80% [4]. Respiration is considered to be improved with age, as thoracic malformations become less pronounced but impairment of the respiratory system remains as a restriction pattern in spirometry [1, 4]. Young patients also suffer from recurrent pulmonary infections during neonatal period or infancy [1, 2].

Renal problems, which include secondary hypertension, polycystic kidneys, pelviectasia, and hypoplasia, exist in 34% of patients and 38% of those with renal involvement may develop end-stage renal disease (ESRD) [1, 2]. These complications are usually found after the second year of life [4].

Liver is impaired in < 30% of cases with ATD [1]. Common findings are prolonged neonatal jaundice with elevated direct bilirubin, elevated liver transaminases, hepatomegaly, polycystic liver disease, and portal hypertension [1, 5]. Histologically, portal fibrosis and bile duct proliferation which may lead to cirrhosis have been reported. Ursodeoxycholic acid administration appeared to control progression of liver disease [5].

Pancreatic and retinal complications are also noticed. The presence of fibrosis and cysts in the pancreatic parenchyma may obstruct normal exocrine function. Retinal dysplasia and retinitis pigmentosa have been described in 15% of patients [1, 2, 4]. The syndrome may...
Ultrasound diagnosis of recurring Jeune’s syndrome: a case report

Ultrasound diagnosis of recurring Jeune’s syndrome: a case report

also be correlated with ciliopathy, malrotation, and Hirschsprung disease. Spinal cord stenosis and C1-C2 compression are life-threatening, although rare complications [1, 2]. Partial agenesis of corpus callosum (1.6%), situs anomalies, and cardiomyopathy have also been reported but they are rare complications [1].

Genetic heterogeneity exists in ATD patients. The main genetic mutation is located in a locus at chromosome 15q13 with a negative mutation analysis for genes GREMLIN and FORMIN1. Furthermore, a mutation in the intraflagellar transport 80 gene on chromosome 3 indicates that ATD may belong to the ciliopathy group. Identifying a cytoplasmic dynein 2 heavy chain mutation (DYNC2H1) on chromosome 11q in five families with ATD or SRP type III supports this finding [1, 2, 4]. Another heterozygous mutation in INVS (NPHP2) gene found in a patient with ATD strengthens the thought of the heterogeneity of the disease [1].

Prognosis of the syndrome is difficult to be made because of the variability of its manifestations [2]. Pulmonary and renal function tests could have a prognostic value as long as RDS and ESRD are the main causes of death [1, 2, 4]. It is reported that only 20% of patients survive beyond infancy [2].

Poor prognosis of the syndrome and low quality of life of these patients is the reason that prenatal sonographic diagnosis is important. An accurate diagnosis can be made between 19-35 weeks of gestation [6]. Although, there have been reported cases with early prenatal diagnosis at 14th and 16th week in both high- and a low-risk pregnancies, respectively [7, 8]. It is suggested that confirmed antenatal diagnosis is possible at the first-trimester especially in high-risk pregnancies because the risk of recurrence is as much as 25% in skeletal anomalies inherited in an autosomal recessive pattern [9]. Most evaluated measurements are femur length (FL), biparietal diameter (BPD), head circumference (HC), thoracic circumference (ThC), and abdominal circumference (AC).

Table 1. — Sonographic characteristics and differential diagnosis [7, 9, 10].

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case Jeune Syndrome</th>
<th>Ellis van Creveld</th>
<th>SRPS I</th>
<th>SRPS II</th>
<th>SRPS III</th>
<th>SRPS IV</th>
</tr>
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<tbody>
<tr>
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<td>Reported increase</td>
<td>Reported increase</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>FL (centile)</td>
<td>&lt; 5° (33.6 mm)</td>
<td>&lt; 5° (21.8 mm)</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>HC</td>
<td>Normal (229.3 mm)</td>
<td>Normal (165.9 mm)</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Thorax</td>
<td>Long and narrow, small, short ribs</td>
<td>Narrow, short ribs</td>
<td>Small</td>
<td>Small, short ribs</td>
<td>Small</td>
<td>Small</td>
</tr>
<tr>
<td>Visceral Anomalies</td>
<td>Pancreatic cysts</td>
<td>Cardiac anomaly, posterior fossa cyst</td>
<td>Hydrops</td>
<td>Exomphalos, bladder outflow obstruction, hydronephrosis</td>
<td>Polydactyly</td>
<td>Urogenital anomalies, hydronephrosis</td>
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<tr>
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<td>Postaxial polydactyly</td>
<td>Tibia</td>
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<td>Autosomal Recessive</td>
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<td>Autosomal Recessive</td>
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</table>

Sonography at 24 weeks gestation showing shortness of all long bones (below fifth centile).

Figure 1. — Thorax and abdomen.

Figure 2. — Femur.
The major sonographic findings are shortness of all four extremities with a rhizomelic pattern, long and narrow thorax and postaxial polydactyly in some cases. Biometric parameters are best-evaluated when expressed as Z-scores[6]. In cases of ATD, polyhydramnios is an often sonographic finding [3]. Increased nuchal translucency (NT) has recently been associated with Jeune’s syndrome case but it is not yet confirmed as a key point to sonographic diagnosis [7]. Pancreatic cysts were found during an ultrasound examination at 15 weeks of gestation in a pregnancy with Jeune’s syndrome [10]. Differential diagnosis of the syndrome must exclude intrauterine growth restriction, chromosomal abnormalities, and especially syndromes such as Ellis-van Creveld syndrome, Verma-Naumoff syndrome, and other short-rib polydactyly syndromes (SRPS) [3, 7] (Table 1).

References


Benign pelvic metastatic leiomyoma: case report

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Summary

Benign metastasizing leiomyoma is a rare condition characterized by benign soft tissue tumors most frequently involving the lung, and is usually associated with a benign leiomyoma or intravenous leiomyomatosis of the uterus. We present a case of a 58-year-old female patient with abdominal pain and symptoms of urinary tract infection four years after hysterectomy due to uterine fibroid. The results of CT revealed a pelvic mass. Pathological examination confirmed that it was a metastatic pelvic benign metastasizing leiomyoma (BML). BML only involving the pelvis is extremely rare. The patient underwent surgical resection and recovered well.

Key words: Benign metastasizing leiomyoma; Pelvic cavity; Intravenous leiomyomatosis.

Introduction

Benign metastasizing leiomyoma (BML) is an uncommon condition characterized by the presence of multiple soft-tissue tumors most frequently affecting the lung [1]. Rare cases involving other anatomical locations including the heart, lymph nodes, omentum and mesentery, bone, peritoneum, pelvic cavity, soft tissue and skeletal muscles of the back and chest wall, breast, or brachial plexus have also been reported [2]. Although cases of BML affecting both lung and pelvis have been documented, to the best of our knowledge, a case involving only the pelvis has yet to be reported. A case of metastatic pelvic leiomyoma is presented.

Case Report

The patient was a 58-year-old female, admitted to the Department of Urology at our hospital on August 4, 2011 with the complaint of two-weeks of right abdominal pain exacerbated in the previous four days, and urinary frequency/urgency, and urodyinia for three days. The patient started experiencing lower abdominal distending pain without obvious cause for half a month. The pain gradually exacerbated with increasing frequency four days prior to the admission. Urinary frequency, urgency, and urodyinia also started three days before the admission. Computed tomography (CT) scan at the outpatient unit revealed a mass in the right ureter. The patient was admitted to the department of urinary surgery in our hospital with the diagnosis of a right ureter unknown mass.

Physical examination showed right side lateral abdominal tenderness (+), right ureter tenderness (+), and bladder area percussion (+). The patient had undergone hysterectomy and oophorectomy due to uterine fibroids confirmed by pathology in 2007 at the Beijing Maternity Hospital. The diagnosis upon admission to our hospital was right ureter unknown mass/urinary tract infection. Doppler ultrasound (US) exam of the urinary tract system showed right kidney hydronephrosis, and extension of the upper section of the right ureter. Pelvic US indicated 3.7 × 3.3 cm hypoechoic mass with unclear border at the right pelvis. Pelvic CT showed a 3.76 × 3.39 cm solid mass (tumor) in the right adnexal area compressing the distal end of the ureter and causing hydrocele above a section of the ureter, pelvis and kidney (Figure 1). Magnetic resonance imaging (MRI) showed a 4.11 × 4.27 × 4.32 cm mass in the right adnexal area, full dilation of the renal pelvis and ureter caused by obstruction of the right lower ureter near the entrance to the bladder (Figure 2). On August 11, 2011 cystoscopy under local anesthesia was performed. No apparent signs of tumor mass in the bladder and ureteral orifice were identified. On August 17, 2011 the patient was transferred to the Department of Gynecology with the diagnosis of metastatic leiomyoma. On August 23, 2011 exploratory laparotomy was performed under epidural anesthesia. An adhesion between the ileum, colon, bladder and pelvic tumor were identified. A firm tumor without clear border about 4 × 4 cm in size was found surrounding the ureter at the entrance to the bladder with strong adhesion to both the bladder and vagina. A sample of tumor tissue was taken for rapid frozen pathology showing benign tumor with focal calcification. The patient then underwent resection of the pelvic tumor, partial ureter, ureteral bladder anastomosis, vesicostomy, and enterolysis. Postoperative pathological exam showed pelvic leiomyoma, partially actively growing, with hyaline degeneration and focal calcification (Figure 3). The patient was discharged 20 days after the surgery and has continued to be followed. She is in good condition and has returned to normal work. The report was approved by the hospital ethics committee and consented to by the patient.

Discussion

BML, intravenous leiomyomatosis (IVL) and leiomyomatosis peritonealis disseminata (LPD) are three unique growth patterns of uterine smooth muscle tumors. BML is a rare lesion affecting females with a history of uterine leiomyomata. In 1937, Steiner reported the first patient who died from multiple uterine leiomyoma. At the autopsy, well-differentiated smooth muscle nodules were found in the lung, therefore the concept of benign metastatic smooth muscle tumor was introduced [3]. There were several hypotheses regarding the origin of the lesion: a benign uterine tumor spreading through the hematogenous route to the lung or other organs as the majority of BML patients have had prior myomectomy or hysterectomy; it could be a low-grade leiomyosarcoma metastasiz-
ing to the lung; or primary pulmonary leiomyomatosis unrelated to but accidently coexisting with uterine leiomyomata [4].

Most BML patients are either asymptomatic or show only mild symptoms, such as slight cough, chest pain, dyspnea, and fatigue, if there is metastasis to the lung. In our case, the tumor surrounded and compressed the patient’s ureter, which resulted in hydronephrosis and secondary infection, and the patient was admitted to the Department of Urology with the symptoms of abdominal pain, urinary frequency urgency, dysuria.

The characteristics of BML remain controversial. Most researchers tend to agree that it is a benign lesion. Disease progression is slow. Most patients have either no clinical symptoms or only mild symptoms, and have been identified accidentally during physical examination. Patients can fully recover after appropriate endocrine treatment or surgical resection of the tumor. For postmenopausal women, the tumor either grew slow or self-resolved. However, some researchers questioned that the pathological examination of uterine leiomyoma reported so far lacked details, such as insufficient sample collection, no record of karyokinesis; or the degree of malignancy could not be determined due to the limitations of currently available pathological tests. Therefore, they speculated that BML might originate from the uterine smooth muscle tumors with unknown malignancy, possibly between benign and low grade of malignancy [5].

For histological origin, most of the literature reports that the lesion is metastasis of a benign uterine leiomyoma to the lung or other organs. The evidence supporting this viewpoint include: 1) from the pathomorphological perspective, metastatic tumors and primary uterine tumors share similar histomorphological and immunohistochemical features, and all are hormone-dependent [6]; 2) Kayser’s research [7] of agglutinin histochemistry in 10 BML cases showed that the majority (80%) of BML expressed galectin-1, galectin-3 and their binding sites; only the expression of galectin-8 demonstrated individual variation, which suggested that these tumors had a common origin; 3) Compared with uterine leiomyoma, pulmonary BML also have similar patterns of androgen inactivation allele [8]; 4) Primary uterine leiomyoma and lung BML also have the same molecular genetic changes – the same X-chromosome inactivation pattern, proving that lung BML is metastasis of uterine leiomyoma, and they are the same clone [8-10]; 5) The results of molecular biology research, including CGA repeat polymorphism detection and analysis of X chromosome activity, showed both tumors have chromosome
lymph nodes are the most common sites of metastases. Since most BML cases also had the history of uterine curettage, myomectomy or hysterectomy, it was suggested that the tumor might be disseminated by surgery [12]. For the patient in our report, the tumor was located at the right ureter, close to the entrance of the bladder. It was a nodular mass 5 × 6 × 6 cm in size, grayish ash in color with interlacing structure on the section. Histopathological findings were leiomyoma with adiponecrosis, degeneration, small cysts, and focal calcification. There was no significant nuclear atypia, mitotic activity or necrosis. Immunohistochemistry showed positivity on vimentin, EMA, SMA, actin, and desmin, which proved its origin of smooth muscle, and the tumors were also estrogen and progesterone receptor positive. The patient had had surgery for uterine leiomyoma four years before. Upon reviewing the pathology slide, there was no significant difference in histology between a pelvic metastatic tumor and primary uterine leiomyoma. Given the pathological findings and location of the tumor, it is likely that the metastasis was the result of surgery.

The diagnosis of BML relies mainly on pathology. For women of childbearing age who have a history of myomectomy or hysterecctomy, the findings of nodular and diffuse lesions of the lung or other organs may suggest this disease. The first consideration is whether the primary tumor is malignant. Sometimes, the malignant area is limited; incomplete sample collection may lead to misdiagnosis. Sometimes well-differentiated leiomyosarcoma has been diagnosed as a tumor alive with cells. Therefore, only when the possibility of malignancy has been ruled out, can the spread and metastasis of benign tumors be confirmed. Some researchers have suggested positron emission tomography-computed tomography examination to identify hidden metastatic lesions [13, 14].

Since the report of BML is limited in the literature, currently there is no standard treatment. The main treatment strategy is the surgical removal of the tumor as completely as possible. Because tumor cells express estrogen, hormone therapy has also been attempted using anti-estrogen drugs such asRaloxifene, LHRH or GnRH-a, but efficacy varied, and for some cases, despite strong expressions of estrogen, progesterone, there was no significant treatment effect [16]. During the postoperative follow-up, our patient did not receive any other treatment, and she has now returned to normal work, without any discomfort. We will continue to follow her.

**Conclusion**

BML is a rare, slow progressing disease, occurring mostly in women of childbearing age who have had history of surgery due to uterine leiomyoma. Lung and lymph nodes are the most common sites of metastases. BML only involving the pelvis is extremely rare. We reported a case of pelvic BML who underwent surgical resection and recovered well. She will continue to be followed.

**References**

Pyomyoma after dilatation and curettage for missed abortion

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Summary

Infection of a leiomyoma is a very rare clinical entity called pyomyoma. Pathology may be encountered during the reproductive period, pregnancy, and even postmenopausal period. In this report, we present a case of pyomyoma which developed after dilatation and curettage managed by broad spectrum antibiotics and myomectomy to preserve the fertility in a 31-year-old patient.

Key words: Pyomyoma; Leiomyoma; Dilatation and curettage; Abortion.

Introduction

Pyomyoma is a rare complication of leiomyomas characterized by infarction and secondary infection of a leiomyoma. Most cases occur during pregnancy or postmenopause and in the literature, only one case has been reported after uterine instrumentation [1, 2].

A case of pyomyoma that developed after dilatation and curettage (D&C) for missed abortion is presented and the course and treatment of a 31-year-old patient are discussed.

Case Report

A 31-year-old primiparous woman was admitted to our outpatient clinic with fever, vaginal discharge, and abdominal pain. She had an intramural leiomyoma and D&C was performed for missed abortion at the ninth gestational week which was three days before. She had a fever (38.5°C) and tachycardia (110 beats/min). Pelvic examination revealed purulent vaginal discharge and uterine tenderness. On transvaginal sonography (TVS), the endometrium was 17 mm thick and there was a 70 x 76 x 80 mm intramural leiomyoma located at the fundus including patchy anechoic zones. White blood cell count (WBC) and C-reactive-protein (CRP) were 23000/μl and 345 mg/dl, respectively. Magnetic-resonance-imaging (MRI) revealed necrotic foci inside the leiomyoma (Figure 1a, b). Cultures were obtained and the patient was started on 900 mg of clindamycin tid and of gentamicin 120 mg/day. The signs, symptoms and laboratory findings did not regress and the endometrial thickness on TVS increased to 20 mm on the 3rd day of therapy. To rule out retained gestational material, D&C was repeated; however, minimal material was obtained and sent for culture. No clinical improvement could be observed until the 5th day and the culture of D&C material revealed Enterococcus faecalis. The antibiotic regimen was adjusted accordingly and 500 mg of imipenem/cilastatin qid and 500 mg of azithromycin bid were started. The patient underwent computed tomography (CT) examination, which revealed abscess loci inside the leiomyoma (Figure 2).

Surgical treatment was decided and the patient was informed about the procedure in details. A Pfannenstiel incision was performed with general anesthesia. There were dense adhesions between the uterus and abdominal viscera. Adhesions were dissected free and an 8 cm intramural leiomyoma located in the fundus was detected. Myomectomy was performed. The leiomyoma was necrotic, and purulent material and debris spilled out (Figure 3). The endometrial cavity was accessed and a drain was inserted from the myomectomy locus transvaginally. An abdominal drain was also inserted.

Fever, WBC count, and CRP regressed dramatically. Histopathologic examination indicated an abscess formation inside the leiomyoma and cultures revealed Enterococcus faecalis. The patient was discharged on the fifth postoperative day with oral antibiotics. Gynecologic examination at the first-month control with TVS revealed normal-sized uterus and normal endometrium lining. The patient reported having regular menses at follow-up and has been symptom-free for more than eight months.

Discussion

The primary pathophysiology in the development of a pyomyoma is inadequate vascularization followed by secondary infection. Leiomyomas can be infected by bacterial seeding of necrotic foci in case of vascular insufficiency (diabetes, hypertension, atherosclerosis) or pregnancy due to hemorrhage and necrosis [2]. The pathogenic organisms are diverse and include both aerobic and anaerobic bacteria such as Clostridium species, Staphylococcus aureus, Streptococcus hemolyticus, Proteus species, Enterococcus faecalis, and S. agalactiae [3]. In our patient, Enterococcus faecalis was cultured.

The most frequent symptoms in case of pyomyoma are fever and abdominal tenderness. However, the condition may be fatal and present with pelvic peritonitis, sepsis, and endocarditis [3-5]. Diagnosis is difficult and imaging methods such as TVS, MRI, CT aid in the diagnosis. The main findings would be heterogeneous echo patterns and cystic spaces inside the leiomyoma [4].

Standard treatment is broad spectrum antibiotics and surgery. When preservation of fertility is intended, simple myomectomy is performed; otherwise hysterectomy needs to be considered [4].

In conclusion, pyomyoma following D&C is a very rare condition. With advanced imaging tools, the pathology can be diagnosed and treated promptly, otherwise the course may be detrimental.
Pyomyoma after dilatation and curettage for missed abortion

Figure 1. — Magnetic resonance imaging revealed necrotic foci inside the leiomyoma (a, axial view; b, lateral view).

Figure 2. — Computerized tomography revealed abscess loci inside the leiomyoma.

Figure 3. — Appearance of the excised infected leiomyoma material.

References


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The management of fusion of the labia minora pudendi in adult women using a radiosurgical knife

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Summary

Fusion of the labia minora is a rare event in adult women and rarely seen as a clinical entity. We present a case of a 30-year-old woman with labial fusion, unmarried, a virgin, and sexually inactive with the complaint of discomfort during menstruation and difficulty in micturition since her puberty. The labia minora were fused in the midline with a pinpoint aperture for draining urine, while the clitoris could not be visualized. Previous history regarding a causal factor was perineal trauma in childhood. Effective surgical resection to separate the fusion was done by a radio frequency surgical knife. After the surgical separation, it was possible to expose the normal hymen behind the previously fused labia. We have shown that the separation of labial fusion in adult women with a radio frequency surgical knife, associated with topical application of estrogen cream, prevents re-fusion of the labia and leads to healing without scarring of the vulva.

Key words: Labial fusion; Perineal trauma; Radio-surgery.

Introduction

Labial fusion is a condition defined as partial or complete adhesion of the labia minora. Adhesions between two labial folds as a clinical entity can also be called: labial adhesion, vaginal synechiae, adhesive vulvitis of the labia, and labial agglutination, occlusion of the vulvae, vulvar fusion and vulvar atresia. This clinical condition usually affects children (three months to six years). It has been reported to occur in up to 1.8% - 5% of prepubertal girls. The peak of incidence is between one and two years of age [1].

Labial fusion is not a congenital lesion and the possible causal factors are thought to be inflammation, irritation due to rash, low estrogen status, nonspecific vulvitis, and repeat urinary infections. All of these can cause denudation of the labial superficial layer, which in turn heals by fibrosis which causes adhesions [2, 3]. The other most common causal factor of labial adhesion is long-term health consequences of female genital mutilation (FGM) [4]. Labial adhesions in childhood can often resolve spontaneously and the resolution has been reported in many cases within one year of diagnosis. The primary treatment for labial adhesions in infants is topical application of estrogen cream to the labia minora [5]. However, following the initial successes, nearly 20% of patients successfully treated with estrogen cream experienced a recurrence of labial fusion [6]. The surgical approach usually consists of an incision and sharp dissection through the fused line and primarily closing with thin sutures [7].

Case Report

In November 2009, a 30-year-old female was admitted to the reproductive unit at our clinic. She complained of discomfort during menstruation and difficulty in micturition since her puberty, and was also concerned about the unusual appearance of her external genitalia. She was unmarried, a virgin, and sexually inactive. This was the first time she visited a gynecologist. She confided to us that her mother told her that she suffered a trauma in the genital region in childhood, when she was less than five years old. Her complaints and discomfort during menstruation and difficulty in micturition had been present since her menarche. The onset of thelarche and pubarche had been normal at the age 11. Menarche occurred when she was 12.5 years of age. This patient had normal female secondary sexual features. Levels of gonadotropins, estrogen, and androgen were normal. Genital inspection revealed a normal mons and hair distribution, but the whole introitus was covered with a very thick skin fusion spreading between the labia majora. In the midline, there was a pinpoint hole measuring approximately 5-7 mm in diameter. The clitoris could not be visualized (Figure 1a and 1b). On rectal examination, the uterus was found to be normal in size, anteroverted, and uneventful. Bilaterally the adnexa were normal. In the Douglas cul-de-sac and vaginal fornix a small amount of fluid was palpated, but the patient did not complain of any discomfort or pain. Transabdominal ultrasonography (US) with a full bladder was performed two or three days after her menstrual bleeding stopped. US revealed a normal anteroverted uterus 12 x 9 x 6 cm in size and the uterine cavity was filled with some quantity of hypoechogenic content. The right ovary was found to be 29 x 22 x 10 mm in size, while the left ovary measured 30 x 25 x 14 mm. The US of the Douglas cul-de-sac indicated that there was some quantity of free-flowing fluid. In the upper third of vagina there was also some quantity of flowing fluid. The patient was admitted with the diagnosis: Total vestibular vulvar atresia, Fusionis labialis minora pudendi. Hematocolpos. Considering how the thick adhesions occluded the vaginal vestibule so completely, we believed that the best treatment would be surgical repair under general anesthesia, separating the fusion and opening the vaginal vestibule by a radio frequency surgical knife (SURGICAL RF UNIT, 150A, 4 MHz/5.8MHz, South Korea). Under
The management of fusion of the labia minora pudendi in adult women using a radiosurgical knife

General anesthesia and after adequate preparation of the operative field, incision was performed initiating from the upper aperture, cutting through the middle line, with the guidance of a metal dilator passed towards the lower end of the fusion. In order to have an adequate surgical result with almost no bleeding and minimal thermal damage of the tissue, we used 3.8 MHz radio frequency. The adhesions at the clitoris were also dissected and the clitoris became visible. At the end of the procedure, the vaginal vestibule was visible with the intact hymenal ring and urethral aperture. At the end of the operation an amount of hemolyzed blood was drained through the hymenal ring and the operative field was not bleeding (Figure 1c).

The cutting edges were not reapproximated with the suture but were left free. Immediately after the operation, vaseline - antibiotic gauze (Stanicid gauze 10 x 10 cm, Hemofarm) and estrogen cream (Dienestrol cream 0.01%, Ortho) were applied to the wound. This topical treatment was continued over the following five days. The postoperative period was normal. On the fifth postoperative day the patient was discharged. She was

Figure 1. — a) Thick skin fusion covering the whole introitus spreading between the labia majora covering the entire clitoris. b) The pinpoint hole in the upper part of the fusion (shown by a dilator) for draining urine. c) Operative wound at the end of the procedure. d) External genital organs one year postoperation.
advised to apply estrogen cream directly with her fingers twice a week, without dressing the wound, and to apply an ointment of Hyperol (Hyperol cream, Meditop, Hungary), every other day for the next two weeks. Two months after surgery at the medical check-up, it was evident that the wound healed per primam and that it was completely covered with young skin. Appearance of the external genital organs seemed to be almost normal. At a further check-up in December 2010 the genital organs appeared completely normal, without evidence of scar tissue formation. Furthermore the patient was not complaining of any difficulty in micturition (Figure 1d). The patient was happy and satisfied with the surgical result and in the end, sought medical advice on the most suitable contraceptive method she could use.

Discussion

Labial adhesions are usually easily and simply diagnosed by physical examination and medical history based on symptoms. The symptoms of labial adhesions in children are most commonly manifested by the dribbling of urine after going to the toilet, or in some cases there may be some vulvar soreness after urinating. The inner lips are joined together, while the perineal region is usually painless although some patients report some vulvar soreness. The exact cause is unknown, but it is strongly suspected that labial adhesions are caused by irritation to the external genitals, though it is not a congenital lesion [8]. If treatment is necessary, based on symptoms or parental request, estrogen cream is indicated. The primary treatment of labial adhesions is application of estrogen cream directly on the labia minora. Estrogen vaginal cream is applied to the adhesions two to three times daily for two to four weeks [9]. The success rate of topical estrogen intervention in girls with labial adhesions is typically about 90%, with published success in case series reports ranging from 46.7-100%. The use of steroid betamethasone (0.05%) cream has also been described. Once the labia separate, an emollient or antibiotic ointment is applied three to five times a day for several months to allow complete healing and prevent recurrence. Recurrence of labial adhesions is a common complication and has been reported in as many as 11.6-14% of cases [9].

The spontaneous resolution and high percentage of successfully treated patients with labial adhesions early in childhood ensure that this clinical condition rarely presents in adult women [10]. In addition, labial adhesions can occur under the influence of factors related to non-specific or specific inflammatory skin conditions, poor local hygiene or absence of sexual activity [11, 12]. The symptoms of labial adhesions in adults are most commonly manifested by difficulties with micturition, menstruation, and sexual activity. Patients usually come to gynecological consultation because they recognize, by personal examination, that the organs are unusual. The external genital organs of these patients show normal mons and hair distribution, but the whole introitus is covered with a thick skin fusion spreading between the labia majora. The physician should check that other genital abnormalities such as imperforate hymen, do not cause difficulties. This clinical condition in reproductive women is very uncommon, especially if it is caused by perineal trauma in childhood [12]. We have presented a case of a 30-year-old woman with labial fusion. She had a trauma in the genital region in childhood when she was less than five years old. She had had a history of discomfort during menstruation and difficulty in urination since her puberty. Because the thick adhesions occluded the vaginal vestibule so firmly, we decided that the best treatment would be surgical repair under general anesthesia. In these clinical conditions, separation of firmly fused labia minora in an adult patient, is usually performed by classical surgical knife or using electro dissection. Simple surgical resection, however, may cause re-fusion and scar deformities [13]. Using electro dissection, the thickness of the thermal damage zone correlates with the power used and the speed of the electrode movement.

Radio frequency surgical knife compared to conventional electro surgery is performed at much lower frequencies (about 0.3 MHz), has less lateral heat spread during the cutting procedure, and results in a thinner thermal damage zone. These were the two main reasons why we decided to perform separation of the fusion and to open the vestibule using a radio frequency surgical knife at a frequency of 3.8 MHz. By this approach, we got straight edges and there was no excess tissue. The cutting edges were not reapproximated with the suture and the edges were left free, which is in contrast to the way this operation is usually performed. Immediately after the operation, the wound was covered with vaseline-antibiotic gauze (Stanicid gauze 10 x 10 cm, Hemofarm) and estrogen cream (Dienestrol cream 0.01%, Ortho) was applied. Covering the wound immediately after the operation with vaseline-antibiotic gauze and estrogen cream ensured that the wound healed by covering with young skin without scarring [14].

This was confirmed two months after the surgery at the medical check-up. It was observed that the operative field was covered entirely with young skin and neither scars nor the deformity of the vulva were visible. The appearance of the external genital organs seemed to be almost normal.

Conclusion

The surgical approach and management of severe labial fusion in adult women by a radio frequency surgical knife was proven effective. Covering the wound immediately after operation with vaseline-antibiotic gauze and estrogen cream prevents refusion of the labial and leads to healing without scarring of the vulva.

References

The management of fusion of the labia minora pudendi in adult women using a radiosurgical knife


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A case report and literature review

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Introduction

Intraperitoneal hemorrhage from spontaneous rupture of uterine varices during pregnancy is uncommon [1]. Its diagnosis is not easy because of its rarity, but also due to the lack of specific signs [2]. It should be considered in any unexplained collapse in pregnant women. The clinical symptoms are stereotypes, however it is associated with acute abdominal pain and shock. Other “classic” causes of hemoperitoneum should of course be mentioned [1]. Exploratory laparotomy is immediately needed to reduce fetal mortality and high risk of maternal morbidity [2]. Through this observation, the authors wanted to emphasize the need to perform Doppler of uterine veins during monitoring of pregnant women to detect uterine varicose veins.

Case Report

The patient was primiparous and 33-years-old. She was at 35 weeks of a twin pregnancy when consulted for remarkable pelvic pain in the last 24 hours. At admission she also complained of fatigue and a feeling of nausea. Conjunctivas were stained and hemodynamic was regular (BP: 130 / 80 mmHg, Pulse: 96 pul / min).

The clinical review found her with uterine contractions and the fetal heart rate recording was normal. On examination, the patient did not describe any bleeding, or uterine trauma. She reported only a throbbing pain in the right flank, as well as several episodes of vagal malaise. The authors then talked about a premature birth and the inevitable removal of the uterine cerclage wire was performed. Minutes after admission there was sudden bradycardia. An emergency cesarean section was then decided associated with in utero-fetal reanimation. Upon admission to the operating room, there was a sudden and unexplained collapse with severe maternal hypotension (70/40 mmHg) and profuse sweating and agitation. Resuscitation was then performed with blood transfusion and macromolecules. Laparotomy revealed an abundant hemoperitoneum (1,100 ml) associated with many clots (Figures 1 and 2). After aspiration, exploration denoted active bleeding from a ruptured varicose package on the right-hand edge of the uterus. Cross-segmental hysterotomy allowed for the extraction of viable twins in apparent good health. Afterbirth and uterine contractions were immediate. No abruptio placentae or uterine rupture were found. After hysterohapy, the authors provided hemostasis of the ruptured varicose veins with several “X” stitches and a hemostatic dressing was applied. Blood pressure was good (140/90 mmHg) at the end of the intervention. The patient was admitted to intensive care for 12 hours. The postoperative course was simple and the patient was discharged on the fifth postoperative day. The newborn male showed signs of brain suffering with diffuse seizures during hospitalization. The evolution was rapidly favorable and electroencephalogram monitoring on the fourth day was satisfactory. The newborn female by comparison had a rather favorable course. Psychomotor development of these children is to be assessed.

Discussion

Frequency

Hemoperitoneum during pregnancy by rupture of uterine varices is an unclear complication because rare and is infrequently described [1]. The present is the only recorded case in decades of the authors’ practice. In the literature, very few authors make reference to it and the first cases were described by Hodgkinson et al. [3]. It concerned mostly primiparous women (55%), apart from any labor in 60% of cases [1]. Closer to home, only nine cases were reported of which seven heifers in the case described by Pitton et al. in 2000 [2]. The predominance of primiparae seems fairly clear such as described in the present case.

Pathophysiology

The pathophysiology of such vascular rupture is still obscure [1]. Several hypotheses have been put forward to try and explain it. According to Hodgkinson et al. [3], the
rupture would occur under the combined effect of hypertension in the uterine veins and their physiological distension in the third trimester of pregnancy, particularly related to their role as a reservoir [2]. It would then be linked to several contributing factors or triggers including postural changes, uterine contractions [3], as well as the posterior insertion of the placenta [2]. In addition, the fineness of vein walls in late pregnancy, unlike those of the arteries, are associated to physiological hypertension and are at risk of rupture [4].

The authors are in agreement with Pittion et al. [2] that the rupture of varicose veins is related to a localized vascular ectasia, which is under the combined effect of hypertension and physiological triggers such as abdominal hypertension (uterine overdistension of the twins).

The formation of uterine varices appears to fall within the general framework of venous pathology in pregnancy and in particular of venous insufficiency. Changes in venous hemodynamics in pregnant women are partly due to compression of the inferior vena cava and iliac vessels by the gravid uterus. This results in an increase in pressure and venous distensibility associated with a slowing of blood flow. The result is the delicacy and fragility of the walls through atrophy of the muscular layer [1].

**Diagnosis**

The differential diagnosis of ruptured uterine varices is for most authors not easy due to the absence of specific signs. However it must be suspected in front of unexplained hypovolemic shock in pregnancy. If the disease is exceptional, the fact remains that many similar cases have been described that highlight the stereotyped nature of the clinical picture. The patient complains of abdominal pain of sudden onset, spontaneous, well-localized in the first instance and then more diffuse. Peristalsis may be present but not evident. There is no reported abdominal trauma, but often a syncopal episode or vagal syndrome [1] as illustrated by this observation. Sudden and unexplained state of shock with no vaginal externalized bleeding is to be expected. The clinical symptoms suggest a uterine rupture in cases of scarred uterus or placental abruption in front of a uterine contraction [2].

Faced with an array of pre-eclampsia, capsular rupture of the liver can be suspected as well a traumatic rupture of the splenic vein.

In front of maternal collapse and fetal distress, it is necessary to refer briefly to such a diagnosis due to hemo- peritoneum and / or shock in order to preserve both maternal and fetal prognoses. Any delay in diagnosis and treatment can be of serious consequence especially in an African limited medical context.

Also among the observations reported to date, only intraoperative findings allowed for the diagnosis of uterine varices, as in the observation described. Often diagnosis is only made intraoperatively, as intervention in this case, during the cesarean section performed for shock and / or acute fetal distress that is generally attributed to a placental abruption or uterine rupture.

In addition to the varices, there are several other diagnoses that coincide with hemoperitoneum during pregnancy aside from traumatic causes:

- the obstetric causes are uterine rupture, placenta increta or percreta, retrouterine hematoma, characterized by acute fetal distress and abdominal pain;
- spontaneous rupture of the liver [5] that occurs in an array of pre-eclampsia and is accompanied by sudden pain shifting to the right upper quadrant and epigastrium;

**Treatment**

Whatever the diagnostic hypothesis mentioned, exploratory laparotomy is immediately necessary due to maternal collapse and fetal distress and will establish diagnosis and proper treatment. Conservative treatment is most often recommended, but caution must be taken to improve the vital prognosis of the patient. Because of the gravity of the hemorrhagic response, some authors have even resorted to hysterectomy [1].

In the presented case, after cesarean section, the authors ensured hemostasis of the ruptures of varicose veins with many “X” stitches. For other authors, hemostasis was achieved by simple points [2, 7, 8]. In the current case, the conservative approach was largely due to stable hemodynamics during surgery and to the speed with which hemostasis was achieved as experienced by...
Pitton et al. [2] in 2000. However, cases of hysterectomy were reported in the literature [1]. This shows the seriousness of the unpredictable situation for the prognosis.

**Prognosis**

Most cases described are far more dramatic than the present, with many examples of in utero fetal death [1]. The maternal mortality rate is high (49.3%) for women not in labor and 76.3% for parturients which have been reported in the literature by Hodgkinson et al. [3]. Other authors have found a maternal death in 28 cases registered and nine perinatal deaths due to breast collapse itself [1]. The maternal and fetal prognosis is better in the presented patient because of early surgery and maternal and neonatal reanimation.

The obstetrical future of these patients is indeed not easier to identify compared to Western literature [2] as sub-Saharan Africa context is less medicalized where patients are lost at discharge. However, the authors believe that patients should be provided with the same advice regarding healthy living rather than suffering from venous insufficiency of the lower limbs: avoid prolonged standing, elevate the legs when lying down, resting in lateral recumbency on the left, the fight against obesity, and regular physical activity [1]. Furthermore, the use of color Doppler could help diagnose the presence of uterine varices in these patients with or without a history of uterine varices. This practice is not widespread in the context of Africa, where its use should be more popularized.

**Conclusion**

Uterine varices in the third trimester of pregnancy are a cause of hemoperitoneum and is rarely mentioned in the literature. It is therefore necessary to keep this complication in mind because the diagnosis and prompt management determine the maternal and fetal prognosis.

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Laparoscopic myomectomy of a giant myoma

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Summary
We present the case of an infertile woman with a giant myoma which was laparoscopically removed. A 34-year-old patient was referred to our department with a large abdominal mass. Ultrasound revealed an 18 cm uterine myoma. Diagnostic laparoscopy showed a giant uterine myoma and with the help of a bent angle camera we started myoma enucleation. The myoma was totally enucleated and removed without disturbing the endometrial cavity. The uterine defect was closed with an absorbable suture in two layers. The myoma was removed using a PK (Gyrus) morcelator, without tissue or blood spillage in the abdomen. The operation time was 165 minutes and the myoma’s weight was 1,200 g. The patient recovered uneventfully. Laparoscopic myomectomy can be an option even for giant myomas, with the condition of an expert surgeon and appropriate surgical instruments.

Key words: Laparoscopic myomectomy; Giant myoma.

Introduction
Laparoscopic myomectomy is a minimal invasive method for management of myomas in women of reproductive age. It offers several advantages, such as shorter hospitalization, faster recovery, fewer adhesions, and less blood loss [1-3]. The main problem is that advanced laparoscopic technical skills are required to perform laparoscopic myomectomy [4]. As a result the procedure is used more frequently for small- and medium-sized myomas [5-8] and there are only a few series of myomectomies for large myomas [9, 10]. We report the case of a 34-year-old patient with a giant myoma who underwent total laparoscopic myomectomy.

Case Report
A 34-year-old nulliparous patient was referred to our department with a large abdominal mass. Bimanual examination revealed a mass which extended from the pelvis up to the abdomen. Abdominopelvic ultrasonography showed an uterine myoma measuring 18 x 10 cm in size. The sonographic appearance of the mass aroused no suspicion of malignancy and the serum concentration of CA-125 was within normal limits.

Written informed consent was obtained from the patient and diagnostic laparoscopy was performed under general anesthesia. The patient was placed in a modified lithotomy position. A foley catheter was inserted and no uterine manipulator was used. Carbon dioxide was insufflated through a Veress needle through the Pulmer’s point. The first assistant held a bent angle camera for better surgical vision in his left hand and grasping forceps in his right (Figure 1). The operator enucleated and removed the myoma without disturbing the endometrial cavity and the uterine defect was closed with an absorbable suture in two layers with intra corporeal knots (Figure 2). The myoma was then removed using a PK (Gyrus) morcelator, without tissue or blood spillage in the abdomen (Figure 3).

The operation time was 165 minutes and the myoma weighed 1,200 g. There was no need for blood transfusion intra- or post-operative and the estimated blood loss was 200 ml. The patient was mobilized the first postoperative day and the recovery was uneventful. The pathology report confirmed uterine myoma and the patient was advised that in case of a future pregnancy she should undergo a cesarean section.

Discussion
Today there is a delay in the age of first pregnancy and consequently uterine preservation is necessary in even more cases of symptomatic myomas including large myomas. Laparoscopic myomectomy using pneumoperitoneum is considered a procedure that requires experienced laparoscopic surgeons [11] and therefore it is not used widely for large myomas. Even though laparoscopic myomectomy offers a better cosmetic result, faster recovery and less postoperative adhesions, disagreement still exists concerning the usefulness of laparoscopic myomectomy for large myomas. It is suggested that the myomas size should not exceed 8 cm [12, 13]. There are reports that bigger myomas result in increased operative time and blood loss, and their cleavage is more difficult [14].

In this article a case of a very large myoma is presented which was successfully removed laparoscopically. As effective enucleation of the myoma is the most crucial step, a bent camera was used in order to improve the surgical vision because the myoma occupied the whole abdominal cavity. This camera afforded the opportunity to enucleate the myoma without bleeding, using bipolar diathermy. Special care was taken to minimize any thermal damage by coagulating under direct vision. There was no excessive blood loss, although this is considered a major problem for large myomas [15]. It is very important for the surgeon to have the appropriate instrumentation. A smart bipolar diathermy offers the best
hemostasis by coagulation, minimizing the thermal damage on the uterus. Moreover the bent camera gives the opportunity to surgically approach areas that the surgeon would not be able to reach with a conventional camera.

The uterine incision was repaired in two layers for better integrity of the myometrium and hemostasis [11, 16]. A 2-0 absorbable suture performing intra corporeal knots was used. The final result was very satisfactory and the patient was advised to undergo a cesarean section in future pregnancy.

The operative time was 165 minutes and most the time was consumed for the removal of the myoma with a PK (Gyrus) morcelator. This morcelator has the advantage of no tissue or blood spillage in the abdomen, preventing from future adhesions and ensuring a better fertility result. It should also be mentioned that laparoscopy results in fewer adhesions than laparotomy with vertical incision which was our other choice.

The patient recovered uneventfully and was discharged the third day after the operation. She needed only paracetamol as painkillers and did not complain of any significant postoperative abdominal discomfort.

This satisfactory result can be explained by the advantages of laparoscopic myomectomy performed by an experienced surgeon. We believe that this is the best choice for fertility preservation even in cases of large myomas considering the other methods that have been proposed, such as mini-laparotomy [15, 17] or gasless laparoscopic myomectomy [18-21].

However further studies on extensive series are needed to better define the indications and long-term results.

References


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