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ORIGINAL ARTICLES

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Introduction

Ovary is a sexual organ generating and discharging ovum, as well as secreting steroid hormone. The occurrence, development, and decline of ovarian function are the foundations in women's whole life stages, which reflect the process beginning from embryo formation to the aging. Correct assessment of ovarian function is significant for evaluating the potential reproductive ability and predicting the age of menopause, as well as providing both individualized and proper treatment and preventive care based on physiological characteristics of women in different phases. Ovarian reserve (OR) is used to predict the potential fertility of women by evaluating the follicles and the quantity and quality of eggs. Currently, there are multiple indexes used to evaluate ovarian reserve, including anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), estradiol (E2), inhibin B, antral follicle count (AFC), etc. Although some scholars combine multiple indexes to evaluate the ovarian function, these indexes are far less accurate, detailed, and comprehensive. To find an ideal method for evaluation of ovarian reserve is the hotspot in research of reproductive endocrine. The present authors, for the first time, put forward a classification system of ovarian reserve function after summarizing numerous cases. It can both accurately and effectively evaluate the ovarian function quantitatively. It is of great help in making clinical decisions and of great significance in future development.

Review

There are multiple indexes already used to evaluate ovarian reserve, widely recognized by most of the researchers in reproductive endocrinology. The currently utilized indexes and their application values are mentioned below after comprehensive analysis of documents.

Age

Age is a factor that has extremely intimate relationship with fertility. The older a woman becomes, the greater the incidence of infertility becomes, and thus fertility competence reduces along with age. It will lead to the decreasing rate of pregnancy and increasing rate of abortion, prolonged average interval between bearing and higher odds of ab-
normal chromosome in next generations, as well as descending live birth rate. It was recorded that the fertility ability of women begins to decrease at the age of 30 years and dramatically decreases at the age of 38 years thereafter towards menopause. This phenomenon is called folded stick mechanism [1]. According to (WHAS11), 90 percent of follicles have been lost by the age of 30, and only three percent of them still exist at 40 years. Recently, researchers found that serum AMH decreased by a rate of 5.6% every year, and the decreasing rate for AFC (2–10 mm) was 4.4%, for the volume of ovary it was 1.1%. There is an evident negative correlation between the age of women and the level of serum AMH, AFC, and the volume of ovary. It has also been found that the proportion of small follicles in ovary (2–4 mm) gradually decreased as the age increases, whereas no obvious changes were seen in the proportion of medium and large follicles, which had the reversed tendency compared with the small follicle. It is rather conspicuous that the increase proportion of large follicle is related to the decreasing level of serum AMH and overall level of AFC. Compared with the actual biological age of women, the age of ovary has stronger connection with the quantity of large follicle given the statistics of AMH and AFC. In other words, it is inaccurate to judge OR merely by the age and the synergy of AMH and AFC has relatively higher accuracy to help predict OR [2].

Ovarian changes and evaluation of ovarian function

The first clinical representation of reproductive senility is the shortened menstrual cycle. Irregular menstrual cycle is the beginning sign of menopausal transition. Stages of reproductive workshop (STRAW) is the first international standardized staging system [3], however, it is restrained only in the changes of menstruation and does not take other influential factors into account, not to mention other prerequisites for this system, such as “only suitable for women older than 40 years with a body mass index (BMI) < 18 or > 30, women without smoking habit and chronically irregular menstrual only, “women with excision of uterus or abnormal anatomy of ovary and uterus are not compatible”. An ideal staging system ought to indicate the normal pattern of menopausal transition, and roughly estimate the time before the final menstrual period, and eventually indicate a warning point (or phase) incorporating other factors like hormones. The standard for this system should be objective, reliable, economical, and convenient, while offering promising prospective and clear boundary. In addition to the aforementioned requirements, cohort studies and statistical analysis validation are also waiting to be implemented, given that the standard of STRAW system is just a consensus for now.

There was a research conducted in 2005, studying the changes of menstruation of middle-aged females in England and measuring the daily discharge amount of four urinary reproductive hormones. It was of significant advancement in this research not only because of the observation in the changes of menopausal transition, but also for the adding of the observation in the changes of sexual hormone. It fully revealed the variation rules of menstrual cycle and reproductive hormones during late period of childbearing, early, and late stages of menopausal transition, so as to help us accurately understand the complexity in the changes of normal menstruation. The present author highly recommends the large sample research conducted by Professor Lin Shouqing, whose student He Zhong published his doctoral dissertation in 2008: “The reminder value of the changes in menstruation to the process of decline of ovarian function”, which proved that the STRAW system can be an accurate indicator for the decline of ovarian function, meanwhile, possessing the disadvantages of incomprehensive staging, unable to reflect endocrine function, especially changes in FSH and AMH. Overall, the change of menstruation is an issue with complex manifestation and difficult statistics.

After a series of clinical and endocrine research, there are four stages generally recognized in the women’s reproductive decline: 1) increasing level of FSH in early follicle phase signals the beginning of reproductive decline; 2) emergence of irregular menstrual cycle indicates the beginning of menopausal transition (MT); 3) absence of periodical pattern in ovarian activity, occasionally, fluctuation of E2 and subsequent uterine bleeding can be expected; 4) cease of ovarian function. The level of FSH and LH continue to upsurge and that of E2 and progesterone continue to drop, indicating menopause. The introduction of new developments in the field of immunology and genetics are conducive to the understanding of the occurring mechanism of ovarian decline, as well as guide clinical work much more efficiently [4].

Hormone and cytokines and the functional evaluation of OR

AMH

The change in AMH levels has been found to be an index that both sensitively and stably assess the function of OR in recent years, it is of unparalleled significance and clinical value. AMH is secreted by the granular cells in pre-antral follicle and small antral follicle, acting during the period when primordial follicle converts to growing follicle, and during the collection of FSH-sensitive follicle in early antral follicle stage. It either directly or indirectly influences the development process of follicle by exerting on AMH receptor, and is capable of inhibiting the growth of follicle and preventing the follicle from growing too rapidly and untimely depleting, and thus has the ability to reserve OR.

Compared with the variation of AFC, there is no significant variation of AMH among different periods of relatively stable menstrual cycles [5, 6], for which characteristic could make AMH a much more convenient
clinical marker compared to AFC, basic FSH level and the ratio of FSH/LH, it also can be measured randomly in one menstrual cycle and free from the restriction of the cycle [7-10]. However, according to the recent studies in which 82 serum samples of 12 women with regular OR underwent serological testing, indicated that there were changes in AMH during the different stages of menstrual cycle. Peak average concentration of serum AMH was 7.9 pmol/L and minimum value was 6.7 pmol/L [11].

According to other researches, there was a downward trend for AMH along with the increase of age. There is a swift drop between 30 and 40 years of age, after which it decreases more gradually. AMH cannot be traced in the women with premature ovarian failure (POF) or menopause [12, 13]. Tremellen et al. [14] observed the level of serum AMH in 238 women ranging from 18 to 46 years of age, with a FSH all below ten U/L. They found that the level of AMH could be maintained at a relatively balanced level of 20–25 pmol/L for women between age of 18 to 29, and began to drop after age 30, reaching ten pmol/L at the age of 37. Surprisingly, no evident change of AMH levels was found between 29 and 37 years of age.

In conclusion, AMH can be used as an ideal index for evaluating ovarian function, foreseeing the decline of ovarian function and predicting the age of menopause [15, 16]. AMH < 0.8 ng/ml can be viewed as severe deficiency of OR. Gnoth et al. [17] studied the indicative effect of AMH on the decline of ovarian function, finding that if they regarded 1.26 ng/ml as the cut-off point, the predictable OR had a decreasing rate of 97% and the hypoergia of ovary could be 88%.

The level of serum AMH not only can be of great importance for evaluating ovarian function, but also can be a brilliant prophet to one’s fertility. Yarde et al. [18] found that the level of AMH had a clear correlation with live birth rate, especially for low-fertility women with increasing FSH. For this reason, it can be an important index for predicting actual fertility. Whereas the other experiments of predicting OR and biological age have restrained value in predicting live birth rate. The live birth rate of the woman with serum AMH above 5.7 ng/ml is 3.18 times of that of others. Regression analysis showed that AMH is a reliable factor to predict IVF [19].

It would be a more reliable index to predict OR if we combined AMH and AFC. The patients with poor ovary response or with no response tend to have high cycle cancellation rate (>22%) and low rate of pregnancy (poor response: 6.7% and no response: 9.8%) [20]. The level of serum AMH is closely related to the number of primordial follicle in ovary, (r = 0.72); there still exists a significant connection between them even after adjustment of age factor (r = 0.48) [21]. Moreover, women with polycystic ovary syndrome (PCOS) have a higher level of serum AMH than healthy one do. For women between 29–38 years of age, the sensitivity and specificity of PCOS is 74% and 79%, as relatively indicated by AMH level and the cut-off value is 3.5 ng/ml [12].

**INH B**

INH B is an important dimer glycoprotein hormone, which belongs to transforming growth factor (TGF) β superfamily. It is mainly secreted by the ovarian granular cells. The principal factor relevant to reproduction is INH, and the subunit of β can be divided into two types: INH A and INH B. INH B is produced by the small primary follicle and secondary follicle, and there is a positive correlation between small AFC in ovary and the basis INH B, whose concentration in serum can reflect the quantity and quality of follicles. In the normal menstrual cycle, INH B reaches its peak in the early or middle follicular phase; selective feedback inhibits the synthesis and secretion of FSH in anterior pituitary, as well as blocks the hypothalamus GnRH so as to stimulate the release of hypophysis FSH. It has a strong negative effect on the secretion of FSH and is good for the maturation of follicle. When OR decreases, INH B produced by granular cells begins to reduce at first; feedback mechanism results in the increase of gonadotropin hormone (GTH), and the decrease of INH B occurs before the increase of FSH and E2. As a result, INH B can be more sensitive than basic FSH and E2 in predicting the function of OR. When INH B ≤ 45 pg/ml, the sensitivity regarding its prediction of low reaction of ovary is 92.9%, and its specificity is 97.4%. Positive predictive value is 86.7%, indicating a drop of OR [22].

There also exists a significant correlation between AFC and the level of serum INH B (r = 0.40) [21]. The level of serum INH B is able to reflect fertility as well. Several studies had shown that fertility dropped along with the decreasing level of INH B [19].

**Baseline FSH (bFSH)**

bFSH (between the second and third day in menstrual cycle) is a commonly used index to evaluate the function of OR. Clinically, FSH ≥ 15–20 mIU/ml is a criteria of low ovarian function [4]. Other hormones like E2, P, LH, etc., also become modified but later than the upsurge of FSH, and their boundary is also ambiguous. Therefore, the change in the FSH leve is an outstanding marker to evaluate the decline of ovarian function. The upsurge of FSH is the early sign of anovulation of ovary and decreasing OR functions. Early studies thought that when bFSH ≥ 151 U/L, the cycle cancellation rate increased, and the peak value of E2 at injection day, collectable follicles, the number of transplanted embryos, and the rate of pregnancy became obviously decreased. The level of serum FSH showed a close relationship with primordial follicle count (r = 0.32) [21].

**FSH/LH (luteinizing hormone)**

The value of FSH/LH has been valued as an index to evaluate the decrease of OR in clinic [23, 24]. Barroso et al. found that patients with FSH/LH > 3.0 had significantly fewer mature oocytes and lower implantation and pregnancy rates than patients with FSH/LH < 3.0 [25]. Peng et
al. [26] thoroughly discussed the connection among 2,721 cycles of FSH/LH values and the consequence of controlled ovarian hyperstimulation (COH), finding that FSH/LH > 2 always means unsatisfied result of patient reacting COH. Lin et al. [27] assessed the value of FSH/LH in normal FSH women, similarly, they found that the ovarian reaction with FSH/LH > 2 was inferior to that of FSH/LH < 1. Shrim et al. [28] conducted a research in patients under age of 41 and with FSH < 8 IU/L, and found that the peak value of E2, the number of obtained eggs and the rates of fertilization and pregnancy was higher than that in the control group. Hence the value of FSH/LH can be used as an index to evaluate ovarian reaction.

Level of E2

Serum E2 mainly derives from granular cells and directly reflects follicular development. Certain E2 levels will guarantee the activation of the hypothalamus-hypophysis-ovary axis, however, an upsurge of basis E2 often indicates the drop of OR. Scientists originally thought that when the basis E2 ≥ 45 pg/ml, OR, the number of developing follicle, the rate of harvesting eggs, pregnancy and fertilization rates were all reduced. It was very difficult to achieve a pregnancy when basis E2 ≥ 75 pg/ml. The cycle cancellation rate could be 33% when E2 ≥ 80 pg/ml, which impeded pregnancy. However according to other reports, there was no evident change in the level of E2 between pregnant and non-pregnant groups.

Smotrichtm et al. [29] proved that regardless of FSH level, fertility could be determined to be low when E2 ≥ 80 pg/ml at the third day of the menstrual cycle. During the process of ovulation induction, the cycle cancellation rate will increase because of low or null reaction of ovary. When E2 ≥ 100 pg/ml, cancellation rate was higher showing worse reaction of ovary. Frattarelli et al. [30] observed 2,634 women, whose E2 levels were either < 20 pg or >100 pg/ml. They separated groups every 10 pg/ml and observed the rate of obtaining eggs, pregnancy, and cycle cancellation. Finally, they reached the conclusion that when E2 < 20 pg/ml or > 80 pg/ml, cycle cancellation rate became obviously increased. It could predict E2 level in women older than 40 years; however, in woman with no cycle cancellation, E2 cannot predict their ovarian reaction and rate of pregnancy.

Ultrasonic testing of ovary and evaluation of ovarian function

Assessing OR by ultrasonography is being increasingly applied for its unparalleled advantages, such as free from trauma, repeatability, and so on. The major processes include assessing AFC, calculating ovarian volume (OVVOL), and stromal blood flow. Antral follicle is the precursor of mature follicle and is a follicle with the diameter of two to ten mm by ultrasound. As the development of follicle does not rely on GnRH stimulation during early stage of antral follicle, large amounts of AFC whose number can perfectly reflect the remnant primordial follicles in the follicle pool, will be mature after enough stimulation by GnRH [31]. With regards to the selection of AFC threshold point, reports have incongruous results. According to Frattarelli et al. [32], patients with AFC ≤ ten had low reactivity towards Gn stimulation and increasing rate of cycle cancellation, but nothing changed in the rate of pregnancy. However in the study of Ng et al. [33], the cycle cancellation rate was 3.8% and 9.1% when AFC ≤ nine and AFC ≤ six. According to Saleh et al. [34], patients with AFC ≤ ten required extra days of stimulation and increased amounts of Gn. Their rates of pregnancy were still low. Domestic study used long protocol to retrospectively analyze 5,865 cases of IVF/ICSI-ET, and indicated that AFC was a good indicator to evaluate OR function, and was better in predicting ovarian reactivity than predicting the result of IVF [35].

Chen et al. [36] believed that for women whose AFC ≤ ten with an age older than 38 or whose AFC ≤ seven could be viewed as the threshold for evaluating the decline of OR. Other scientists defined the types of ovary by the amount of AFC (unilateral), less than five mean decline of OR, 5–12 follicles indicated normal, and higher than 12 was considered polycystic ovary. AFC with 2~6 mm diameter could be more effective to reflect OR [37]. The small follicle (2~4 mm) reduced along with increase of age, no evident changes for middle follicle (5~7 mm), and the large follicle (8-10 mm) increased along with the increase of age. The increasing proportion of large follicle is closely related to the reduced AMH and overall AFC [38]. AFC and the number of primordial follicle have significant correlation with each other (r=0.78). There still exists significant connection between them even after adjustment for age factor (r=0.53) [21].

It was reported that the predictive value of AMH and AFC exceeded that of FSH, E2, and INH B [19]. AUC was 0.935 and 0.905, respectively; the sensitivity was 93% for AFC evaluating low reaction of ovary and specificity was 88%, AMH sensitivity was 100%, and specificity was 73% [2]. In addition, AFC is a reliable factor to evaluate live birth rate of IVF and important element to assess one’s fertility [39].

The volume of ovary and number of follicles are closely related to OR function; the decreasing volume of ovary and decreasing number of antral follicles indicates the decline of OR function. However, the diagnostic value of ovarian volume index is controversial, as its normal volume is between 2.47~7.75 cm³, which is of great variation. If the threshold is 2 cm³, the volume of the ovary has decreased before the upsurge of FSH. The volume of ovary is the independent predictive factor of the number of acquired oocyte; its sensibility and specificity are equally 75% [40]

At present, there are different kinds of indexes used in the study of mesenchymal blood of ovary, such as peak systolic velocity (PSV), pulsation index, resistance index, vascularization index, discharge index, etc.; however, all cannot be easily compared with each other. In light of the
connection between mesenchymal blood and OR or IVF reactivity, we can predict its potential in the prediction of menopausal age and OR evaluation. It can be classified as 0–III. Experts thought that except for the age factor, when the PSV > ten cm/s, the number of mature eggs and rate of pregnancy were kept at a relatively high level [40]. Therefore, the performance of mesenchymal ovarian blood before promoting ovulation could be used as an index to reflect OR function. As a result of lacking researches, a more accurate measure is required to establish a more accurate normal range [41]; furthermore, it is very difficult to perform clinically, so further studies are needed to assess its application value.

### Immunity and function evaluation of ovary

Several studies had shown that POF was related to autoimmunity. The connection between autoimmunity and OR is drawing more and more attention. Studies from molecular immunology indicate that 4–10% of POF patients have lymphocytic oophoritis. Because of lacking sufficient evidence and more precise diagnostic tools to determine the pathogenesis (autoimmunity), the detection of all relevant antibodies (autoimmunity) from POF patients is lacking. Currently, most believe that anti-thyroid-globulin antibody (TG-Ab), anti-thyroid peroxidase antibody (TPO-Ab), and anti-Adrenocortical antibody (ACA) can be used to perform immunological detection for OR, as well as offer immunological treatment; thus we could ameliorate incipient ovarian failure and transitional ovarian failure and even get our patients recovered from POF completely [24, 41]. Researchers have made remarkable progress in clinical treatment for the POF patient with positive TG-Ab. Their theoretical basis is that POF is reversible and the residual function of follicle may be revived after immune dysfunction, which finds more important value in the stage of incipient ovarian failure and transitional ovarian failure.

ACA is the representative of immunity oophoritis. Autoimmune diseases relevant to POF include diabetes, myasthenia gravis, xerophthalmia, autoimmune polyglandular syndrome (APS), etc. ACA is only a warning sign in evaluating OR and has not reached a quantitative criteria.

### The prospective of establishing classification system for ovarian function

It is quite obvious that all the aforementioned indexes capable of predicting OR and reflecting ovarian function cannot sufficiently reflect OR. Therefore research proposed the combination of several indexes to evaluate ovarian function, which is functionally superior to any single index. Oliveira et al. [42] combined the level of serum AMH, number of antral follicles, and age to establish ovarian response prediction index (ORPI). ORPI = (AMH×AFC)/age, 101 cases of ICSI women were involved, and the results showed that there was close connection between ORPI and rate of pregnancy, obtaining eggs and the number of egg during mitosis II; thus, it can be an indicator in predicting ovarian function. This study was an attempt to quantify OR function, however, the authors themselves believed that it was not sufficiently comprehensive, detailed, and objective.

### Classification system for ovarian function

After years of accumulation in clinic and referring to assorted documents, (classification system for fallopian tube function) and (staging of endometriosis by American fertility association), the present authors established classification system of their own based on the currently effective indexes (Table 1).

#### Definition of different grades

- OR grade I is scored 1–2. The OR is basically normal and should be rechecked after half year.
- OR grade II is scored 3–8. It belongs to the menopausal transition (MT) phase. The patients need to be closely observed and offered treatment when necessary. For those desiring to become pregnant, indications will be given in time and assisted reproduction will be implemented when necessary.
- OR grade III is scored 9–10. Most are in the early period of menopause (within two years after menopause). Hormone replacement treatment (HRT) will be given for those with no contraindications.
- OR grade IV is scored over 10. Most are at the late period of menopause (more than two years after menopause). For these women who have osteoporosis and urogenital system atrophy should be closely monitored and given timely treatments. HRT and calcium supplement should be given under strict observation.

#### Classification system indications

- Patients with organic diseases, such as hypoplasia of gonads, ovarian arterial embolism, and gynecologic malignant tumor, etc, should not be included in this classification.
Final confirmation can be reached only after three laboratory reports with at least one month interval. Because the decline of ovarian function in the same individual is complex, hence the examination is needed to be performed at least twice, and even several times in order to evaluate comprehensively.

Although the influence to AMH and AFC is not extensive when taking oral contraceptives, it can reduce FSH, LH, and E2. Therefore ovarian function evaluation is given three months after drug withdrawal. For other steroid hormones used, the evaluation should be performed one month after the drug withdrawal.

Interventional treatment of ovarian cyst is likely to have effects on ovarian function, hence evaluation is given three months after treatment.

**High-risk group**

- The events occur twice or more within six menstrual cycles: the menstrual duration is altered (> seven days or more different with normal menstrual cycle); or menstrual cycle is no more than 21 days (oligomenorrhea).
- Women older than 38 years of age affected with joint and muscle pain, discomfort, fatigue, insomnia or with any unexplained causes.
- Women with autoimmune diseases, such as Sjogren's syndrome, Hashimoto’s thyroiditis with high level TPO-Ab (> 1,000 IU/ml) and rheumatic or rheumatoid arthritis.
- Family history of diabetes or only existence of insulin resistance; although many years after menopause, some of these patients may have higher levels of estrogen accompanied by metabolic syndrome. So the patients should be followed up closely to prevent endometrial lesions and so on.
- Family history of POF.
- At least twice operations history of ovarian or fallopian tube surgeries; hysterectomy performed more than five years ago.
- Patients who suffer anxiety disorder, obsessive-compulsive disorder, and other mental illness requiring antipsychotic drug treatment.
- History of active tuberculosis (TB).

**Conclusions**

The present authors are first to devise the concept of classification system for ovarian function, which is of great significance for the following reasons: 1) quantitative evaluation of ovarian function can be used in guiding clinical diagnosis and treatment; 2) the classification system can be used in instructing family planning; 3) it can be an early warning sign of ovarian decline; 4) it can help in determining the proper time for performing HRT or ET, as well as in evaluating the effect of treatment; 5) effective system is able to predict the menopausal age; 6) this system can instruct how to treat the diseases of the women in perimenopausal period combined with myoma of uterus, adenomyosis, etc. If their ovaries have a high grade of function, they are anticipated to enter menopause in the upcoming one or two years, thus are free from operative treatment; 7) objective system can instruct ART, such as evaluation reaction rate and cancellation rate of ovary, and so on. It is of great help in enhancing pregnancy rate and live-birth rate.

The feasibility of this classification system is waiting to be verified and improved in the future. At present, what the authors have accumulated through clinical assessments has been used in instructing ART and operations. They hope to improve this system step-by-step through continuous clinical practices, making it a more scientifically effective in the foreseeable future. Further studies will be published within in the next two years.

**Acknowledgments**

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**References**


Dental management in pregnancy: recent trends

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Summary
This review analyzes the changes that occur during normal pregnancy and describes the main odontogenic infections, suggesting the actual best approach in dental management. Several studies support the hypothesis that periodontal disease is associated with preterm labour and other conditions complicating pregnancy, such as pre-eclampsia and fetal growth restriction. Appropriate dental care and prevention during pregnancy may reduce poor prenatal outcomes and eliminating risk factors. Dental examination before pregnancy is strongly suggested in order to act early on dental and periodontal diseases. Prevention means reducing the presence of bacterial plaque through professional hygiene sessions, education, and motivation to proper oral hygiene at home, education in proper nutrition, a balanced diet, and low intake of sugars. For these reasons, it is essential to have a more intense interdisciplinary collaboration between gynecologist and dentist in order to achieve an optimal women’s health, during this particular time in their lives.

Key words: Oral health in pregnancy; Preterm labour; Dental care; Odontogenic infections.

Introduction
Pregnancy is associated with important anatomical, physiological, biochemical, and endocrine changes, which affect multiple organs and systems. These changes are essential to adapt woman’s body to the pregnant state and to promote fetal growth and survival. They are largely secondary to the effects of progesterone and estrogens, which are produced predominantly by ovary in the first 12 weeks of pregnancy, and then by placenta. The progressive physiological status that occurs includes changes in the cardiovascular, haematologic, respiratory, renal, gastrointestinal, endocrine, and genitourinary systems.

The hormones induced by pregnancy determine changes in the mother’s body, and oral cavity is not an exception. These modifications in the oral cavity could increase susceptibility to oral infection. Although these adaptations of maternal organ systems are normal, they require consideration and adjustments in treatment by any dentist who is providing oral healthcare and prescribing medications for the patient [1-8].

This review analyzes the changes that occur during normal pregnancy and describes the main odontogenic infections, suggesting the actual best approach in dental management.

Physiologic changes associated with pregnancy
The respiratory tract undergoes important changes as a result of maternal adaptation to pregnancy. During pregnancy, women may experience systemic disorders such as respiratory alterations: dyspnea (in 60-70% of all the pregnant women), hyperventilation, snoring, an upper ribcage breathing pattern and chest widening, and rhinitis [9-12].

Cardiovascular and haematological changes begin as early as at four weeks’ gestation and are progressive: elevation of the coagulation factors V, VII, VIII, X, and XII, and reduction of the factors XI and XIII, with an increased fibrinolytic activity to compensate the increased clotting tendency [13, 14].

Gastrointestinal disorders represent some of the most frequent complaints during pregnancy. An increased intragastric pressure and a reduction in the lower esophageal sphincter tone, which is secondary to inhibition of the production of the motilin peptide hormone (due to a rise in progesterone concentrations observed in pregnancy), determines gastroesophageal reflux disease in 30-70% of all pregnant women, and an almost two-fold prolongation of the gastric emptying time as compared to non-pregnant women [15-20]. Nausea and vomiting (hyperemesis gravidarum) are experienced by 66% of all the pregnant women, commencing at approximately five weeks after the last menstrual period, and reaching a maximum prevalence after eight to 12 weeks [21, 22].

Diarrhea occurs in up to 34% of pregnant women and the incidence rate of constipation in pregnancy is 11-38%. The etiology is multifactorial, with decreased small bowel motility, decreased motilin level, decreased colonic motility, increased absorption of water, and iron supplementa-
The dramatic hormonal and hemodynamic modifications of pregnancy could also alter renal function and these changes should be considered when assessing renal function in pregnancy and in case of medications provided through delivery. As might be expected from the increase in cardiac output, renal plasma flow and glomerular filtration rate during pregnancy increase. An increased renal perfusion, particularly during the second half of the pregnancy, gives rise to an increased drug excretion in the urine [28-30].

Endocrine alterations are also observed in pregnant women. Insulin production rises during pregnancy, but is accompanied by increased insulin resistance caused by placental hormones (mainly human placental lactogen). Maternal hyperglycaemia causes increases in fetal insulin and this can result in neonatal hypoglycaemia as the carbohydrate load falls immediately after birth [31, 32]. Physiologic changes associated with pregnancy here discussed, are listed in Table 1.

### Oral health in pregnancy

In the oral cavity, pregnant women may suffer a series of dental alterations such as caries, gingivitis, periodontal disease, and erosions. This increased risk is mainly due to the increase in cariogenic microorganisms produced by the nutritional changes and to a reduced attention to oral health, coinciding with a drop in salivary pH and buffer effect. These changes in salivary composition are observed in advanced-stage pregnancy and during lactation, and may temporarily increase vulnerability to both caries and enamel erosion [33, 34].

Many scientific studies have shown that saliva has multi-potential effects on oral cavity such as lubrication, anti-microbial effect, buffering, pH regulation, and protection of teeth. Biochemical changes in saliva can lead to oral and dental tissue injury [35, 36].

Female steroid sex hormones influence oral health through different mechanisms. Gingival tissue is affected by hormonal changes during puberty and pregnancy, as shown by more pronounced subclinical signs of gingival inflammation during the ovulatory phase of the menstrual cycle and by the worsening of pre-existing gingivitis during human pregnancy. Pregnancy induces an increased response of the gingival tissues to local factors, such as plaque and tartar, through disturbance of tissue metabolism [37-40].

Gingivitis is the most common oral disorder during pregnancy, with a prevalence of 60-75%, and tends to appear in the second month of gestation. Approximately 50% of all women with pre-existing gingivitis suffer from a worsening condition during pregnancy, as result of the fluctuations in estrogen and progesterone levels, in combination with changes in the oral flora and a reduced immune response. The maximum intensity is observed in the eight month, then gingivitis decreases. Other factors such as accumulation of dental plaque and deficient oral hygiene may be considered as causal or aggravating factors [37, 39].

Periodontitis is a relatively common clinical condition, which occurs in more than 30% of people in some populations; it has a prevalence between 5% and 20% in pregnant women. Treatment in pregnancy is safe and easily applicable and involves scaling and root planning [40, 41]. An association between periodontal disease and preterm birth has engendered much interest and despite advances in obstetric and dentistry care, this particular condition continues to be the leading cause of perinatal morbidity and mortality [42-44].

During the second trimester of pregnancy, the proportion of anaerobic gram negative bacteria increases with respect to the aerobic bacteria in dental plaque [45]. Lipopolysaccharides can activate macrophages and other cells, inducing production and secretion of cytokines, such as IL-1β, TNF-α, IL-6 and PGE2, and matrix metalloproteinases. If these compounds reach the general circulation and cross the placental barrier, the levels of PGE2 and TNF-α in amniotic fluid may increase, and premature delivery may result [38, 44]. This suggestion has led many investigators to seek evidence in this field. Since a relation of periodontal disease with preterm birth was proposed, many observational studies have been carried out. Although the pathophysiological mechanism remains unclear, several studies support the hypothesis that periodontal disease is associated with preterm labour and other conditions complicating pregnancy, such as pre-eclampsia and fetal growth restriction. This association has also been reported by most of the observational studies, which concluded that pregnant patients with periodontal disease have a 2.8-fold increased risk of preterm birth [44, 45].

Other alterations that can appear during this period are: pyogenic granuloma and aphthae, which should be moni-

### Table 1. — Physiologic changes associated with pregnancy.

<table>
<thead>
<tr>
<th>Respiratory</th>
<th>Increased respiratory rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decreased functional residual capacity</td>
</tr>
<tr>
<td></td>
<td>Increased tidal volume</td>
</tr>
<tr>
<td></td>
<td>Increased minute ventilation</td>
</tr>
<tr>
<td></td>
<td>Respiratory alkalosis</td>
</tr>
<tr>
<td>Cardiovascular and haematological</td>
<td>Increased cardiac output</td>
</tr>
<tr>
<td></td>
<td>Increased blood volume</td>
</tr>
<tr>
<td></td>
<td>Increased resting heart rate</td>
</tr>
<tr>
<td></td>
<td>Decreased peripheral resistance</td>
</tr>
<tr>
<td></td>
<td>Decreased blood pressure (2&lt;sup&gt;nd&lt;/sup&gt; trimester)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Decreased gastric motility</td>
</tr>
<tr>
<td></td>
<td>Decreased esophageal sphincter tone</td>
</tr>
<tr>
<td>Renal</td>
<td>Increased renal plasma flow</td>
</tr>
<tr>
<td></td>
<td>Increased glomerular filtration rate</td>
</tr>
<tr>
<td></td>
<td>Increased renal perfusion</td>
</tr>
<tr>
<td></td>
<td>(second half of the pregnancy)</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Increased insulin production</td>
</tr>
<tr>
<td></td>
<td>Increased insulin resistance</td>
</tr>
</tbody>
</table>
tored; salivary changes, including variations in pH and composition; an increased frequency of temporomandibular joint disorders, though these seem to be more related to dental loss, malocclusions or poorly executed fillings during this period [46-48].

**Dental management during pregnancy**

As recommended by the recent guidelines, it is appropriate to undergo to dental examination before pregnancy, in order to act early on dental and periodontal diseases. However, this preventive approach often is not completed because, according to the literature, more than one-third of pregnancies is not planned. After delivery, the patient may undergo certain dental visits and dental treatment, without any maternal and fetal risk [41, 48, 49].

The main position of the patient's chair should be semi-reclined, as well-tolerated, with any change of position every five minutes. Supine position is absolutely not recommended, as it promotes the compression of the aorta and vena cava by the gravid uterus, causing a hypotension syndrome. The sessions should be short (20-25 minutes) and not stressful. The most challenging procedures should be postponed after delivery. A careful medical history and blood pressure control shall be made during the dental examination [41, 47].

Patients should be also motivated to perform correct oral hygiene and go to the dentist periodically [41, 47, 49], in order to reduce gingival inflammatory response to the local irritants usually associated to the hormonal changes observed during pregnancy. In addition, emphasis should be placed on the advisability to reduce the consumption of refined carbohydrates [33, 34, 39].

The use and benefits of fluoride administered during the prenatal period for the subsequent prevention of caries in deciduous teeth is subject of intense debate. Fluoride is clearly able to cross the placental barrier and is absorbed by the fetus, although its true efficacy is not clear. In this context, several studies analyzed the effect of prenatal fluoride upon the incidence of caries in deciduous teeth and on the appearance of fluorosis. When appropriately used, fluoride is a safe and effective agent that can be used to prevent and control dental caries [50-52].

**Treatment modalities**

Fetal organogenesis takes place in the first three months of pregnancy and is very sensitive to external factors (drugs, maternal stress, and irradiation). Over the next six months the fetus grows and becomes less sensitive – though a number of factors can still exert an influence, such as infections or certain drugs such as tetracyclines.

During the first trimester (from conception to week 14) only emergency dental treatment is indicated, avoiding elective dental procedures because of the vulnerability of the fetus. Oral hygiene should be often reinforced with plaque control and tartrectomy, if it is necessary.

The second trimester (from week 14 to week 28) is the safest period for elective dental treatment. It is advisable to avoid X-rays during this period, though if they prove necessary, they should be obtained under adequate safety conditions (beam collimation, high-speed film, filter, lead protection, high kV setting or constant beams, in-use quality program), and only selected periapical or bitewing images should be contemplated in most cases [33, 34, 53]. It is preferable to postpone extensive reconstructions or major surgical procedures after delivery [33, 34].

Regarding drug use during pregnancy, the main concern is the possibility that fetal toxicity or teratogenicity may result if the drug is able to cross the placental barrier. Polytherapy is to be avoided and any necessary prescription should be decided administering the least effective dose for the shortest time possible. In any case, medication should be avoided in the first three months of pregnancy. Before prescribing or administering a drug to a pregnant patient, the dental professional should know the classification of Food and Drug Administration (FDA) for the prescription of drugs to pregnant women according to the risk of fetal damage. This classification contemplates five categories: A, B, C, D, and X [54]:

**Category A**

Adequate and well-controlled studies have failed to demonstrate a risk to the fetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters). Example drugs or substances: levothyroxine, folic acid, magnesium sulfate, and liothyronine.

**Category B**

Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women. Example drugs: metformin, hydrochlorothiazide, cyclobenzaprine, amoxicillin, and pantoprazole.

**Category C**

Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks. Example drugs: tramadol, gabapentin, amlodipine, trazodone, and prednisone.

**Category D**

There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks. Example drugs: lisinopril, alprazolam, losartan, clonazepam, and lorazepam.
Studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits. Example drugs: atorvastatin, simvastatin, warfarin, methotrexate, and finasteride.

In this context it is advisable to prescribe drugs belonging to groups A and B. However, many group C drugs are also administered during pregnancy. During lactation it is necessary to assess the possible risk of maternal drug use for the breastfeeding infant, and to evaluate the possible safer alternatives. If medication proves necessary, however, it should be administered after breastfeeding, in order to facilitate elimination of the drug before the next feeding time and thus minimize exposure of the nursing infant [54].

In order to prevent any problem, dentist should remember that drugs are absorbed easily during pregnancy, as the serum concentration for drug binding is lower than that in the non-pregnant state. There is also a higher volume of drug distribution, a lower maximum plasma concentration, a lower plasma half-life, higher lipid solubility, and a higher clearance of the drugs. All these factors allow an easy transfer of an unbound drug across the placenta, thus exposing the fetus to the drugs. Certain drugs are known to cause miscarriage, teratogenicity, and low birth weight of the fetus [54].

**Conclusion**

As shown above, it is evident how easy and important it is to establish a prevention program to minimize problems during pregnancy. Prevention means reducing the presence of bacterial plaque, through professional hygiene sessions, education, and motivation to proper oral hygiene at home, education in proper nutrition, a balanced diet, and low intake of sugars. For these reasons, it is essential to have a more intense interdisciplinary collaboration between gynecologist and dentist in order to achieve an optimal women’s health, during this particular time in their lives.

**References**

Dental management in pregnancy: recent trends


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Introduction

Uterine leiomyomas (also called uterine myomas or uterine fibroids) are the most common solid benign pelvic tumors in the female reproductive system, causing significant morbidity including pain, pelvic pressure, menstrual disorder, and reproductive dysfunction [1, 2]. Although hysterectomy and myomectomy are the two conventional treatments, minimally invasive techniques have become increasingly accepted as alternative therapeutic modalities for symptomatic uterine leiomyomas. Since the first report from Lee [3] in 2002, radiofrequency ablation (RFA) has emerged as an effective treatment option for patients with symptomatic leiomyomas [4]. Image-guided percutaneous RFA also has advantages over surgery by potentially causing less morbidity and mortality, with reduced cost, and hospital stay.

The authors carried out a systematic review and meta-analysis to assess the efficacy and safety of RFA for symptomatic uterine leiomyomas based on all relevant studies.

Materials and Methods

This meta-analysis was performed according to guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [5]. Two authors (K.J.I. and J.H.K.) independently performed the literature search, eligibility assessment, data extraction, and quality assessment. A third author (D.Y.Y.) was consulted for discrepancies.

Literature search

The authors conducted a systematic review and meta-analysis of studies reporting the use, efficacy, and safety of RFA for the treatment of symptomatic uterine leiomyomas. Scientific articles were retrieved from the databases of Medline, Embase, and Cochrane Library from the time of inception to August 2014, and a search of relevant citations in appropriate articles was performed. Keyword search was conducted using combinations with Boolean operators “OR” and “AND” with the following medical subject heading (MeSH) terms and free-words: (“radiofrequency ablation” or “RFA”) and (“uterus” or “uterine”) and (“myoma” or leiomyoma” or fibroid”). The authors limited searches to publications of human studies reported in English.

Criteria for inclusion and exclusion

Articles were manually selected from the results as they pertained to the treatment of symptomatic uterine leiomyomas with RFA, with a set of predetermined inclusion and exclusion criteria. The study inclusion criteria for this systematic review and meta-analysis were as follows: 1) original articles, 2) cohort of patients aged over 18 years with a diagnosis of uterine leiomyomas treated with RFA, 3) minimum of ten patients treated, 4) study that assessed at least one clinical outcome measure, such as tumor vol-
ume change, symptomatic improvement, procedure-related complication, and reintervention, and 5) clinical and imaging follow-up for at least one month. The rationale for the last of these inclusion criteria was that the majority of complications typically occur during or soon after the RFA procedure; thus, one-month follow-up duration captures a significant proportion of all complications.

Abstracts, letters to the editors, reviews without original data, expert opinions, editorials, supplements, case reports, systematic reviews, and studies in which the outcomes were not clearly reported were excluded from the analysis. Studies that mixed other effective interventions (i.e., uterine arterial embolization, other type thermal ablative therapy, and surgery) simultaneously in the same subject(s) were also excluded. Finally, studies on transvaginal RFA were excluded from this meta-analysis, because there is a basic difference between percutaneous and transvaginal approaches for RFA of leiomyomas. If multiple studies were reported by the same institution and/or authors, the one with the highest-quality data was included in the analysis.

Data extraction

Data were extracted from the entire content of each identified article using standardized forms. Descriptive data extracted from each study included the first author, country, year of publication, study design (prospective or retrospective; there were no randomized controlled trials [RFAs] or comparative studies), number of patients, age of patients, RFA system, and length of follow-up.

The outcome measures included change in tumor volume, change in symptom severity score, change in health-related quality of life (HRQL) score, major and minor complication rates, procedure-related mortality, and reintervention rates. Major complications were defined as those requiring hospitalization for therapy, prolongation of inpatient hospital stay (> 48 hours), or involving permanent adverse sequelae. The reintervention included repeated RFA procedure, uterine arterial embolization (UAE), myomectomy, and hysterectomy. When multiple follow-up data points were available, all post-treatment outcomes for each subject were abstracted from the longest follow-up data, with the exception of complications and reinterventions.

Data analysis

Statistical analyses of selected studies were performed with Cochrane Review Manager (RevMan) version 5.2 and the Comprehensive Meta-Analysis version 2.2. For the change in tumor volume, change in symptom severity score, and change in HRQL score, the standardized mean difference between pretreatment and post-treatment with a 95% confidence interval (95% CI) was calculated. For each meta-analysis, the I² tests were first calculated to assess the heterogeneity of the included studies. Studies without significant heterogeneity (I² < 50 %) were analyzed with a fixed-effects model, and studies with significant heterogeneity (I² > 50 %) were analyzed using a random-effects model to pool the results. In addition, the risk of bias of included studies was assessed by using Cochrane Collaboration’s tool.

Results

Selection and characteristics of included studies

The PRISMA flowchart of the search and selection of studies is depicted in Figure 1. The extensive electronic search led to the identification of 46 articles. After screening the titles and abstracts of the search results, 23 studies were found to be eligible and their full-text publications were analyzed. Of these, 15 full-text articles were excluded, because they did not meet the predefined inclusion criteria. Finally, eight studies published between 2005 and 2014 were included in this systematic review and meta-analysis.

All eight studies were prospective observational studies; no RCTs or comparative studies were found. There were a total of 370 patients with uterine leiomyoma treated with percutaneous RFA. The design and baseline characteristics of included studies are described in Table 1.
up time varied from nine to 36 months, with a duration of 12 months or shorter in six (75%) of studies.

**Outcome assessment**

In the analyses of the effects of RFA on tumor volume, symptom severity score, and HRQL score, there were significant heterogeneity among the studies ($I^2 = 86\%, 70\%,$ and $76\%$, respectively), thus the random-effects model was used to pool the results. The pooled meta-analysis of these data demonstrated a statistically significant change in tumor volume of $-80.96$ ml (95% CI: $-37.07$ to $-124.85$), with follow-up ranging from nine to 36 months (Figure 2). The change in symptom severity score was statistically significant, with a mean of $-42.76$ (95% CI: $-38.22$ to $-47.30$), at 9–24 months' follow-up (Figure 3). The change in HRQL score was also statistically significant, with a mean of $38.34$ (95% CI: $33.02$ to $43.65$) at 9–24 months' follow-up (Figure 4).

**Table 1.** — Baseline characteristics of the studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>First author (reference)</th>
<th>Country</th>
<th>Year of Publication</th>
<th>Study design</th>
<th>No. of patients</th>
<th>Age (years)* of patients</th>
<th>RFA System</th>
<th>Follow-up interval (months)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergamini V. [7]</td>
<td>Italy</td>
<td>2005</td>
<td>Prospective</td>
<td>18</td>
<td>44.3 ± 2.5</td>
<td>RITA model</td>
<td>1, 3, 6, 9, 12</td>
</tr>
<tr>
<td>Ghezzi F. [8]</td>
<td>Italy</td>
<td>2007</td>
<td>Prospective</td>
<td>25</td>
<td>42.2 ± 2.5</td>
<td>RITA model</td>
<td>6, 12, 24, 36</td>
</tr>
<tr>
<td>Carratello G. [9]</td>
<td>Italy</td>
<td>2010</td>
<td>Prospective</td>
<td>11</td>
<td>40.4 ± 6.0</td>
<td>Le Veen coaxial</td>
<td>1, 3, 6, 9, 12</td>
</tr>
<tr>
<td>Garza Leal J.G. [10]</td>
<td>Mexico</td>
<td>2011</td>
<td>Prospective</td>
<td>31</td>
<td>40.2 ± 5.9</td>
<td>Halt 2000</td>
<td>3, 6, 12</td>
</tr>
<tr>
<td>Iversen H. [11]</td>
<td>Denmark</td>
<td>2012</td>
<td>Prospective</td>
<td>46</td>
<td>44.6 ± 7.5</td>
<td>RITA model</td>
<td>3, 6, 9</td>
</tr>
<tr>
<td>Chudnoff S.G. [12]</td>
<td>USA</td>
<td>2013</td>
<td>Prospective</td>
<td>135</td>
<td>42.4 ± 4.7</td>
<td>Accessa system</td>
<td>3, 6, 12</td>
</tr>
<tr>
<td>Robles R. [13]</td>
<td>Guatemala</td>
<td>2013</td>
<td>Prospective</td>
<td>35</td>
<td>43.6 ± 4.7</td>
<td>Halt Medical</td>
<td>3, 6, 12</td>
</tr>
<tr>
<td>Galen D.I. [14]</td>
<td>USA</td>
<td>2014</td>
<td>Prospective</td>
<td>69</td>
<td>42.1 ± 5.5</td>
<td>Accessa system</td>
<td>3, 6, 12</td>
</tr>
</tbody>
</table>

*mean ± standard deviation. Note—RFA = radiofrequency ablation.
The pooled proportion of complication rate for RFA was 10.5% (95% CI: 7.3%–14.9%) (Figure 5). Table 2 summarizes the 27 complications found in the review. The most commonly reported complication was abdominal pain with 16 reported cases (59.3% of total complications). There were five adverse events (including two abdominal wall hematomas, one pelvic abscess, one sigmoid colon laceration, and one vaginal bleeding) that could have been considered major complications. There were no reported deaths as a result of RFA or a related complication. A total of five reinterventions occurred, with the pooled rate of 2.7% (95% CI: 1.3–5.6%) (Figure 6). There were four hysterectomies and a UAE procedure performed across all the studies (Table 3).

### Risk of bias

The risk of bias in this meta-analysis was assessed by Cochrane Collaboration’s tool, and the outcome is shown in Figure 7. The percentages of high risk of bias in "Were incomplete outcome data adequately addressed?" and "Was
the study free from selective outcome reporting?" were both 50%, thus there were attrition bias and reporting bias in this meta-analysis.

Discussion

Although surgical resection remains the standard treatment for uterine leiomyomas, RFA has been accepted as one of the non-surgical treatment options for patients with symptomatic leiomyomas [7–14]. RFA causes tumor cell destruction through the application of a high frequency alternating current that generates high frictional heat leading to protein denaturation and coagulation necrosis [15, 16]. The major advantage of RFA therapy is to destroy tumor cells without damaging adjacent vital structures. In addition, RFA can be performed in a minimally invasive fashion under ultrasound guidance, which is used for accurate localization of the probe within the tumor [16].

Although there are several articles in the literature supporting the application of RFA in the treatment of patients with symptomatic leiomyomas, the available evidence from those studies is weak due to the sparseness of data, disagreements among studies, the limited number of observa-
tions, or the lack of a systematic review. Meta-analysis is an important tool that combines the genotyping data from all eligible published studies and has the advantage of increasing statistical power and reducing random error, thus defining the effect of clinical interventions more precisely [17]. To the authors’ knowledge, there has been no previous comprehensive systematic review or meta-analysis of RFA in the treatment of uterine leiomyomas. Thus, to provide the most comprehensive assessment of the efficacy and safety of RFA for leiomyomas, they performed this meta-analysis in which eight studies were finally included.

The outcomes from the present meta-analysis showed statistically significant improvements from baseline to final follow-up, including reduction in tumor volume, decrease of symptom severity score, and improvement of HRQL score. However, it should be noted that there was no consistency in follow-up durations between studies. Variable length of follow-up durations can affect the degree of tumor volume reduction and symptom improvement.

This systematic review and meta-analysis summarizes the available evidence on complication and reintervention rates of RFA in the treatment of leiomyomas. Although several complications were noted in the included studies, most of them were either easily managed or self-limiting, thus considered minor complications. Abdominal pain and urinary tract infection were the most commonly reported complications. Although the authors did not perform a subgroup analysis on this item owing to the limited data, the forest plot of complication rate of this meta-analysis showed lower rate of complications in more recent studies published in or after 2012 when compared with studies published before 2012 (Figure 5). Possible explanations for this difference are improved selection of patients for RFA, advanced techniques and devices, and greater operator experience in the procedure.

Only one study [9] included in the preset meta-analysis classified specific complications into major or minor categories. According to ‘The Society of Interventional Radiology clinical practice guidelines’ [18], major complications are events that may result in hospitalization for therapy, prolongation of inpatient hospital stay (> 48 hours) or permanent adverse sequelae. Based on this grading system for complications, the authors categorized minor and major complications in this review. Only five major complications of RFA were reported at a rate of approximately 1.4% in this study. However, none of these events resulted in death.

An additional outcome evaluated in this meta-analysis was the rate of reintervention to manage the residual uterine leiomyoma. The results of the present study showed that the pooled rate of reintervention after RFA was 2.7% within nine to 24 months after the procedure. The early reintervention after RFA might be related to the residual tumor tissue, which is mostly caused by insufficient tumor ablation. In cases of uterine leiomyoma in dangerous locations, it is often difficult to achieve curative tumor ablation by securing a specific safety margin in three dimensions. In contrast, late reintervention may be associated with tumor progression after RFA. Accordingly, the relatively short follow-up period in the present meta-analysis (9–24 months) may underestimate the precise reintervention rate.

Currently, there are no meta-analyses comparing the efficacy and safety of RFA and other less invasive therapy for uterine leiomyomas. Toot et al. [19] conducted a meta-analysis consisting of 54 studies for evaluation of the effects of UAE, including major complications and reintervention. In their study, the major complication rate defined by the same criteria to the present study was 2.9% (95% CI, 2.2–3.8%) and the rate of hysterectomy for resolution of a complication was 0.7% (95% CI, 0.5–0.9%). Although not directly comparable, the outcomes of the present meta-analysis indicated that RFA had lower rate of major complication and higher rate of reintervention than UAE.

Some possible limitations in this meta-analysis must be acknowledged. First, the present review mainly suffers from lack of RCTs comparing the use of RFA with other surgical or non-surgical treatment. The ten case series included in this meta-analysis were all observational studies. Second, the reported data in all reported series has been heterogeneous in terms of patient selections, utilization of RFA systems, and definitions of complications. All of these may have led to significant between-study heterogeneity for the outcome measurement. Third, the relatively short follow-up periods and the relatively small number of included patients may make the interpretation of the efficacy of RFA difficult. Approximately half of patients (in seven of ten studies) included in the present meta-analysis had a follow-up duration that is equal to or less than 12 months. The relatively short follow-up period in included studies may result in the potential underestimation of the calculated complication and reintervention rates. Finally, the present authors did not include non-English language publications, which may create language bias. It has been well known that studies with statistically significant (positive) results are more likely to be published in English [20].

Conclusion

In conclusion, the results of this meta-analysis showed that RFA is a safe and effective treatment for symptomatic uterine leiomyomas. However, higher quality clinical trials are needed to identify this outcome and to provide sufficient evidence on the matter.

References

Percutaneous radiofrequency ablation for symptomatic uterine leiomyomas: a systematic review and meta-analysis


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The predictor markers of ovarian response in poor responders under 40 years of age

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Summary

Objective: To explore the ovarian reserve markers in predicting ovarian response and pregnancy rates in poor responder patients undergoing in vitro fertilization (IVF). Material and Methods: A total of 140 women < 40 years with poor ovarian response (POR), who underwent IVF were included in the study. The clinical findings compared with normal responder controls (n = 250). Regression analysis was used to search the correlation between the number of the total oocyte count retrieved and independent variables as age, FSH, LH, AMH, AFC, and E2 on the hCG day. Results: AUC ROC curve were AMH 0.804, AFC 0.701, E2 on hCG day 0.786, FSH 0.705, LH 0.527, and E2 0.479, age 0.707, respectively. E2 levels on hCG day and AMH levels were independent markers of POR. None of the factors were predictor of pregnancy rate. Conclusion: The serum E2 levels on the hCG day and AMH levels predict ovarian response, but not pregnancy rates.

Key words: In vitro fertilization; Anti-Müllerian hormone; Poor ovarian response; Cycle cancellation; Ovarian reserve; Pregnancy rate.

Introduction

The accurate assessment of functional capacity of the ovary before controlled ovarian hyperstimulation (COH) is an essential issue in the success of infertility treatment. Ovarian reserve describes the number and quality of oocytes pooled in each ovary and it declines with an increasing age, resulting in a decrease in female reproductive function [1, 2]. However, chronological age does not always reflect biological age, and they may not always correlate with each other [3]. Various studies have assessed ovarian reserve with different ovarian reserve markers such as age, follicle stimulating hormone (FSH), estradiol (E2), antral follicle count (AFC), and anti-Müllerian hormone (AMH). However, the results are variable depending on the population and markers studied [4-19]. There are some disadvantages of day 3 FSH levels and AFC that are the most common markers used. Firstly, FSH level may not be an accurate marker for ovarian reserve due to cyclic fluctuation of hormone. Also, high FSH values occur late in the aging process. Secondly, the accuracy of measurements of AFC depend on ultrasonographer [6-18]. AMH measurement is the most famous test with promising results. However, the results of the previous studies varied due to heterogeneity of population and laboratories and kits [6-8]. Regarding FSH levels that increased in later phases of ovarian aging, AMH seems to be more valuable and earlier marker of ovarian aging in the studies [6-18].

Poor ovarian response is a sign of ovarian ageing, and it is an important limiting factor in vitro fertilization (IVF) success. Although the incidence is unknown, it is encountered in approximately 10-15% of women undergoing IVF [19]. The delayed childbearing age has increased the rate of poor ovarian response (POR) [20]. There is a paucity of studies until Bologna criteria for POR definition [21]. The Bologna standards define the poor response. The diagnosis performed by existence of two or more of the following features such as advanced maternal age or risk factors for POR, previous POR, and abnormal ovarian reserve test [22]. Introduction of these diagnostic criteria is a significant step toward reproducibility and homogeneity of the studies. Most of the studies among POR include cases with advanced age [7-21]. In this study different from the others, the authors examined women under 40 years of age. This current study aimed to investigate the predictive role of ovarian reserve markers in ovarian response of poor responder patients under 40 years of age.

Materials and Methods

This study was designed retrospectively among women undergoing COH in an Assisted Reproductive Techniques Unit of Kocaeli University. The local ethics committee approved the study. Clinical details of all treatment cycles prospectively entered into a computer, which were retrieved for analysis, retrospectively. A total of 140 patients who fulfilled the inclusion criteria be-
The present authors determined the cut-off values, sensitivity, and specificity of AMH levels for poor response to be 1.10 ng/mL, 80%, and 55.2 %, respectively. The values below the cut-off level were estimated as poor responders.

**Table 1. — The characteristic findings of participants.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Poor responders</th>
<th>Normal responders</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (years)</td>
<td>19-40</td>
<td>32.1±5.2</td>
<td></td>
</tr>
<tr>
<td>Gravida</td>
<td>0-8</td>
<td>0.49±0.98</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>0-3</td>
<td>0.02±0.17</td>
<td></td>
</tr>
<tr>
<td>Abortion</td>
<td>0-6</td>
<td>0.28±0.77</td>
<td></td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>0-3</td>
<td>0.09±0.37</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.2-32</td>
<td>22.2±3.2</td>
<td></td>
</tr>
<tr>
<td>Couple’s age (years)</td>
<td>23-60</td>
<td>35.2±5.9</td>
<td></td>
</tr>
<tr>
<td>Duration of marriage (years)</td>
<td>1-30</td>
<td>7.2±4.9</td>
<td></td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>1-20</td>
<td>6.1±4.1</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. — The comparison of biochemical and hormonal values of poor and normal responders.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Poor responders</th>
<th>Normal responders</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8±4.6</td>
<td>24.2±4.1</td>
<td>0.311</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1±1.4</td>
<td>2.0±1.3</td>
<td>0.872</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>8.7±4.1</td>
<td>7.2±2.5</td>
<td>0.000</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>5.8±3.0</td>
<td>5.4±3.0</td>
<td>0.239</td>
</tr>
<tr>
<td>E₂ (pg/ml)</td>
<td>54.0±39.1</td>
<td>56.1±93.6</td>
<td>0.729</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>1.0±1.44</td>
<td>1.9±1.9</td>
<td>0.000</td>
</tr>
<tr>
<td>AFC</td>
<td>8.8±5.7</td>
<td>12.8±6.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Basal progesterone (ng/ml)</td>
<td>4.5±7.0</td>
<td>8.5±9.4</td>
<td>0.250</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>27.7±13.8</td>
<td>35.1±17.2</td>
<td>0.02</td>
</tr>
<tr>
<td>DHEA-S (ng/ml)</td>
<td>162.1±67.3</td>
<td>213.9±89.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Results

The age of the 390 women ranged from 19 to 40 years with a mean duration of infertility was 6.1 ± 4.1 years. Table 1 presents the characteristic findings. Of the 390 patients enrolled, 140 (35.9 %) patients were poor responders, while 250 (64.1%) women were normal-responders.

Table 2 shows a comparison of biochemical and hormonal findings. BMI, blood glucose, homeostatic model assessment-insulin resistance (HOMA-IR), basal LH, E₂, and progesterone were similar. However, FSH levels were significantly higher in poor responders, while AMH, AFC, total testosterone, and dehydroepiandrosterone sulfate (DHEA-S) levels significantly decreased. Gonadotropin initiation dose, total gonadotropin doses, and cycle cancellation rates were considerably higher in poor responders. The hCG day E₂, LH, metaphase two (MII) oocyte count, and pregnancy rates were significantly lower. Table 3 presents the details of the comparison.

A poor ovarian response was defined as fewer than three oocytes; ovarian reserve markers performed well in the prediction of poor response. The area under the curve (AUC) ROC curve for ovarian reserve markers were AMH (0.804, p < 0.01), AFC (0.701), E₂ on hCG day (0.786), FSH (0.705), LH (0.527), and E₂ (0.479), age (0.707), respectively. Figures 1a and 1b show the results of the ROC curve analysis.

The present authors determined the cut-off values, sensitivity, and specificity of AMH levels for poor response to be 1.09 ng/mL, 80%, and 55.2 %, respectively. The values below the cut-off level were estimated as poor responder.
The predictor markers of ovarian response in poor responders under 40 years of age

populations. The serum AMH cut-off level in patients with cycle cancellation was 0.72 ng/ml with 75% sensitivity and 56% specificity. If AMH was 0.08, 99.8% of cycles were cancelled.

Total number of oocytes retrieved was related to a variety of factors. There was a negative relation to chronological age \( (p = 0.00; \ r = -0.393) \), day 3 FSH level \( (p = 0.00; \ r = -0.302) \). There was a positive relation to AFC \( (p = 0.00; \ r = 0.518) \), E2 level on hCG day \( (p = 0.00; \ r = 0.571) \), AMH level \( (p = 0.00; \ r = 0.529) \). However, the correlation between the total number of oocytes and BMI, insulin level, HOMA-IR, day 3 LH, day 3 E2, day 3 progesterone, and endometrial thickness were insignificant.

Linear regression analysis was used to search the correlation between the number of total oocytes (dependent variable) and independent variables as age, FSH, LH, AMH, AFC, and E2 on an hCG day. According to this model, AMH and an hCG day E2 levels were independent predictors of the OR (Table 4). If FSH level > 10 mIU/ml, AFC < 6, AMH < 0.72 ng/ml were accepted as associates, none of the factors had an independent effect on pregnancy rates.

Discussion

In this study, ovarian reserve markers predicting poor ovarian response in women under 40 years of age were researched. Ovarian reserve determination maintains the optimization of follow-ups of patients undergoing IVF. Optimizing the treatment protocol according to ovarian reserve parameters will lead to optimal gonadotropin doses, adequate protocols, and sufficient information before the start of induction [17]. Although all ovarian reserve parameters related to the oocyte number, only AMH and the hCG day E2 levels were independent predictors of ovarian response, but not PR. The present study has a limitation of retrospective design. However, this study differs from the others that evaluated AMH cutoff values in women under 40 years of age to predict POR.

Several studies have assessed of ovarian reserve with
different ovarian reserve markers [9-25]. Recently, AMH measurements in predicting ovarian response gains priority [2, 7-16]. FSH, LH, AMH, AFC, and E2 on the hCG day were considered as independent variables, and a regression analysis model was used for the oocyte count and pregnancy rates. These results were similar to results of Sahmay et al. [10]. The results of AUC ROC curve analysis for poor ovarian response showed that AUC ROC was 0.804 for AMH, 0.701 for AFC, and 0.786 for E2 on hCG day, respectively. The present authors determined the cut-off level of AMH as 1.09 ng/ml. When the AMH level was below 0.72 ng/ml, cycle cancellation would probably be seen and also in cases with AMH values lower than 0.08 ng/ml, almost all the cycles cancelled. The present results suggested that AMH levels may aid in proper decision-making before stimulation program in women under 40 years of age. Although various studies indicated the predictive value of AMH measurements before COH protocols, there are no standard cut-off values for AMH measurements. Previous studies reported several cutoff values for a POR that ranged from 0.1 to 2 ng/ml: approximately a 20-fold variation exist between the results [13-15]. Since different commercial kits can cause different results from different laboratories, it is difficult to reach a consensus on cut-off values [13-16].

In conclusion, AMH and hCG day E2 levels were independent predictors of ovarian response. In young, poor responders, measurement of AMH before COH protocol was the most sensitive marker to predict ovarian response, but had no effect on pregnancy rates. Despite the measurements of AMH in ART are promising, prospective studies in different age groups are needed.

References


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Colposcopy today

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Summary
A brief digression of the advent and development of colposcopy is described, along with its advantages in the prevention and diagnosis of uterine cervical cancer.

Key words: Colposcopy; CIN; Cervical cancer.

Introduction
The contribution of colposcopy in the prevention and diagnosis of uterine cervical cancer is universally acknowledged, although it was originally only utilized by German physicians or by countries with scientific relations with Germany. Its evolution coincided with the development of more advanced instrumentations and the knowledge of preinvasive or invasive lesions of the cervix.

Colposcopy today
Since Hinselmann introduced colposcopy as a useful tool in the study of the lower genital tract in 1925, the technique has experienced different avatars [1, 2]. Being a German technique, after World War II, its use was limited to those countries under the scientific influence of Germany.

Two pathologists De Brux from France (1967) [3] and Richart from USA, (1967) [4] discovered the real significance of the so-called dysplasias, and demonstrated that it was the same disease with the capacity to progress to carcinoma. Richart introduced a new terminology “cervical intraepithelial neoplasia” (CIN) grades 1, 2, and 3.

National cervical cancer screening programs have been cytology based. More recently, the knowledge of the etiopathogenic role of HPV persistent infection in the development of cervical preinvasive and invasive lesions has added new useful screening tools such as HPV tests. Nowadays the role of colposcopy is relatively well defined as an aid to diagnosis instead of a diagnostic test itself in patients with abnormal cytology in the absence of a grossly visible lesion. This fact has classically limited its practice to experienced colposcopists who work in specialized units, where most will limit the use of the technique by just nearly describing extension and morphology of the lesion. The usefulness of colposcopy should not be limited to the approach of premalignant or malignant diseases of the cervix, because it is a dynamic instrument that allows clinicians to detect, describe, interpret, and even predict severeness and grading of the observed lesions, benign or not, that otherwise would be misdiagnosed or not diagnosed at all by the naked eye. Moreover colposcopy helps in this same way when we talk about vulva, vagina, perineum, and perineal regions. This article is not intended to include a comprehensive listing of all known dermatological or pathological disorders that may be diagnosed by using the colscope in vulva or vagina, but to highlight that there are many common disorders that could be identified [5].

There is also some concern on the fact that postponing the colposcopy to be performed in a specialized unit and the drawbacks that this kind of practice could bring to the patients’ personal (psychologically) and social/working life (due to the need to book two appointments), every time that a patient is encouraged to go to a specialized unit to have a colposcopy, fear and anxiety could affect her, knowing that there is a need for further investigation. As for cost-effectiveness, when colposcopy is part of the routine gynecological examination, there is no extra charge. The expense of a colscope is covered indirectly, including no need of reassurance of the patient in another visit, reduction of naked eye misdiagnosis or underdiagnosis, precise application of topical treatment, and thereby decreasing complications and avoiding insufficient therapy, and patients’ anxiety related to the colposcopy itself is completely reduced and almost nonexistent [6, 7].

When mentioning the uterine cervix evaluation, we also dear to recommend the routine use of colposcopy during the annual gynecological check up. It is quite clear that such proposal implies some problems: increase in the time of the consultation, requires some knowledge of the technique, but also
has some other clear advantages than those stated before: as the technique obliges to introduce the speculum in order to visualize the entire cervix, the number of cytologies without glandular component decreases and the colposcopy would help the clinicians identify if there is any extension of the lesion to the endocervix or vagina. In some cases, as we know the expert colposcopist had diagnosed a severe lesion, and cytology gave a false negative diagnosis [8].

In the recent years, light amplification by the stimulated emission of radiation (LASER) with CO₂ technology has become an important and excellent tool to treat vulvar, perineal, perianal, vaginal, and cervical disorders, because of its major advantages when compared with traditional techniques (scalpel, electrosurgical excision procedures, etc) such as: high degree of clinical efficacy, bloodless field, sparing of normal tissue, rapid healing with minimal scar formation, small number of complications, and microscopic precision due to the possibility to couple the LASER beam equipment to the colposcope. If this technology was introduced to the gynecological field with the intent to remain longer, as it appears to be, colposcopy will be an important partner with LASER energy to achieve this goal. On the other hand, it is quite surprising the rapid spread of ultrasound used by the “general” gynecologist giving a definitive diagnostic, in spite of the imaging specialists that normally have better tools and the responsibility of the accurate diagnostic.

With this article, we as a group with enough experience in colposcopy want to encourage other similar groups and general gynecologists themselves to rethink and argue this great and useful gynecological technique [9, 10].

References


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Introduction

Adenomyosis of the uterus is a common phenomenon among women of reproductive age. Uterine adenomyosis is a pathological phenomenon in which the endometrial glands and stroma invade the myometrium, although the normal growth is intrauterine. The cause of adenomyosis is unknown, and some studies have suggested that certain relative factors, such as implantation, immunity, metaplasia, and genetic factors are related to adenomyosis. Pregnancy and delivery, relapse-induced abortions, and endometritis are closely related to adenomyosis, and women who undergo uterine surgery are at risk for the condition. Adenomyosis is divided into the following types: diffuse and localized, and the latter is characterized as an adenomyoma. Adenomyosis leads to dysmenorrhea, lower abdominal discomfort, menorrhagia, and polymenorrhea, which could cause infertility and spontaneous abortion; some cases could be asymptomatic and difficult to identify from uterine myomas.

Adenomyosis is diagnosed by a postoperative pathological analysis. The adenomyosis diagnosis rate could be improved by evaluation of progressive dysmenorrhea, menstrual disorders, and menorrhagia, and a pelvic examination is efficacious in the diagnosis. Ultrasonography, particularly transvaginal ultrasonography, facilitates the identification or classification of the disease, and improves the accuracy of the diagnosis. In some cases, the features of adenomyosis are miniscule, subtle and non-specific, and pelvic magnetic resonance imaging (MRI) might be necessary. MRI is more advantageous for the diagnosis of uterine adenomyosis because of its excellent soft-tissue resolution and multiple plane imaging. However, the high cost limits the functionality of MRI.

The treatment of adenomyosis is individual and should be based on the age of the patient, the clinical symptoms, and childbearing considerations. Patients with mild symptoms could be treated with anti-inflammatory pain-killers and a levonorgestrel-releasing intrauterine device could be used for the treatment of adenomyoma. Gonadotropin-releasing hormone analogues (GnRHa) are used to relieve severe symptoms, such as dysmenorrhea, lower abdominal discomfort, menorrhagia, and polymenorrhea. GnRHa is more efficient than other drugs and plays a role in the down-regulation of the hypothalamic-pituitary-ovary axis, which could lead to female climacteric syndrome including hot flashes, insomnia, loss of muscle tone, and osteoporosis. GnRHa is more efficient for adenomyosis than other drugs, however symptom relapse occurs after cessation of GnRHa. For the patients with severe symptoms, the definitive treatment plan for adenomyosis has been a hysterectomy. In recent years, social conditions have resulted in more patients selection uterus-saving treatment, for fertility or other reasons.

Adenomyosis is divided into two types: diffuse and loc-
calized, which is characterized by an adenomyoma.

For patients with severe symptoms, ultrasonography and the pelvic MRI facilitate the diagnosis of adenomyoma, and an adenomyomectomy, particularly an abdominal or laparoscopic one, has become possible for those women. Compared with an abdominal adenomyomectomy, a laparoscopic adenomyomectomy has several advantages, including better visualization, less invasion, faster recovery, fewer adhesions, and shorter duration of hospitalization. However, it has not been recommended for widespread use for a number of reasons, such as the difficulty of surgery, as well as the risk of uterine rupture and adenomyoma relapse. The present study aimed to demonstrate the validity, reliability, and security of adenomyosis treatment with a laparoscopic adenomyomectomy.

Materials and Methods

The study included 216 patients with severe adenomyosis, who underwent surgery with a laparoscopic adenomyomectomy between January 2008 and September 2012 in the Department of Gynecology of Second People’s Hospital of Changzhou, an affiliate of Nanjing Medical University. The degree of dysmenorrhea score was based on a visual analogue scale (VAS).

Before each surgery, the patients’ medical history was carefully collected and each patient underwent medical examinations and pelvic examinations. Ultrasonography and the pelvic MRI were used to diagnose adenomyoma in the patients with severe symptoms. The exclusion criteria included size >16 weeks of gestation, and adenomyoma size > 8 cm. An adenomyoma in the posterior uterine wall of one patient is shown by ultrasound, MRI, and laparoscopy (Figures 1-4).

All of the patients who participated in this study did so in the context of mutual trust, open communication, and informed consent. The study received approval from the ethics committee. All the surgeries were performed by Professor Ruxia Shi, with the same nursing team.

Operative procedure

After general anesthesia was administered via an orogastric tube, the patients were placed in the Trendelenburg position. After the abdominal cavity was filled with CO₂, a ten-mm trocar was placed through the umbilicus, and three five-mm trocars were placed in the hypogastrium. A uterine manipulator was placed in the uterine cavity to assist the movement of the uterus.

Pituitrin was injected into the myometrium of the uterus to reduce the risk of bleeding (Figure 5). In the middle of the adenomyoma, a transverse incision was made by the monopolar electrocoagulation through a five-mm trocar. An ultrasonic scalpel and aspirator/irrigator probe were used to help separate the adenomyoma (Figure 6). The aspirator/irrigator probe washes blood in the operating field. The purpose of the surgery was resection of the regional lesions (Figure 7), relief of symptoms, and to recovery function.

The incision in the uterus was sutured continuously by 1-0 vicryl in two or three layers (Figure 8). No dead space and hem-
A novel laparoscopic surgical technique for severe adenomyoma

orrhaging was observed in the myometrium. Then, the adenomyoma was removed by morcellation (Figure 9). During the procedure, attention was focused on the position of the pelvic organs to avoid damage. The pelvic cavity was washed with 1,000 ml of saline to reduce adhesions. All the removed tissue were analyzed by the pathology department.

All the patients had a follow-up evaluation at one, three, and six months, and then at one and two years. The Student’s t-test and chi-square test were applied for the data in this study, and a p-value < 0.01 was considered statistically significant.

Results

In this study, 216 patients underwent a laparoscopic adenomyomectomy. The patient characteristics are shown in Table 1. The mean age of the patients was 37 (± 3.3) years, and the BMI was 26 (± 2.7). In total, 98 patients had a history of pelvic surgeries. The operative and postoperative outcomes are shown in Table 2. In total, 176 patients experienced severe dysmenorrhea, and the prevalence of severe dysmenorrhea in this study was 81.48%. In total, 157 patients experienced menorrhagia and polymenorrhea, with a prevalence of 72.69%. The primary infertility and secondary infertility rate was 44.44% (96/216).

The volume of bleeding during surgery was 150.2 ± 22.7
ml, the mean operative time was 60 ± 48.6 minutes, the mean time for anal exhaust was one ± 1.8 days, and the mean duration of the hospital stay was five ± 1.2 days. During the surgeries, there were no bladder or bowel injuries, and all the surgeries were completed successfully with no conversions to open surgery (Table 2).

The outcome and surgical efficacy of each surgery was investigated at one- to 24-month follow-up examinations. At the six-month follow-up, most patients had good outcomes. The follow-up data dysmenorrhea, menorrhagia, and polymenorrhea are shown in Table 3. Of the 176 patients, 170 (96.6%) showed significant improvements in the symptoms of dysmenorrhea after surgery, whereas six patients had a poor clinical outcome. In 151 (96.2%) of the 157 patients, there was an obvious effect on the menstrual cycle.

Of the 97 patients who wanted to maintain their fertility, 77 (79.4%) had their successful pregnancies by natural conception or assisted reproduction within two years following surgery and 50 (51.5%) patients delivered at term. All of the 50 women were delivered by cesarean section, and 44 patients returned to the present hospital for their operations. Seven (15.9%) patients who returned had severe pelvic adhesions, 22 (50%) patient had mild or moderate adhesions, and 15 (34.1%) had no obvious adhesions.

Discussion

Adenomyosis is a common diseases in women of childbearing age [1]. Adenomyosis is divided into two types: diffuse and localized, and the latter is characterized by the presence of an adenomyoma. In recent years, social developments have resulted in more patients selecting uterus-saving treatment for fertility or other reasons. In patients with severe symptoms, that are not alleviated by a levonorgestrel-releasing intrauterine device or GnRHa, particularly an abdominal or laparoscopic adenomyomectomy is practical for women in whom preservation of the uterus is important. A laparoscopic adenomyomectomy has several advantages, including better visualization, a lower degree of invasion, faster recovery, fewer adhesions, and shorter duration of hospitalization.

Research has shown that laparoscopic adenomyomec-

tomy is seldom used and one reason for this lack of use is the difficulty of identifying the adenomyosis location. To solve this problem, transvaginal ultrasonography was used to identify and locate the adenomyosis for every patient before surgery. In some cases, pelvic MRI has been considered. MRI has more advantages for the diagnosis of uterine adenomyosis because of its excellent soft-tissue resolution and multiple plane imaging. Some reviews of the accuracy of ultrasonography, particularly transvaginal color Doppler sonography (TVCS), and MRI for the diagnosis and differential diagnosis of uterine adenomyoma, showed that ultrasonography and MRI were useful examinations [2]. In the present study, TVCDS, and MRI were used before surgery, which increased the surgical accuracy.

Another limitation on the use of the laparoscopic adenomyomectomy procedure is uterine bleeding during surgery, difficulty suturing, and the pelvic adhesion after the operation. Pituitrin was injected in the uterine myometrium to reduce the risk of bleeding and was used once or twice during the operation, additionally the authors cauterized the uterine incision to reduce the risk of bleeding. In the middle of the adenomyoma, they performed a transverse incision by monopolar electrocoagulation, which was easier to suture. The uterine surgical defect was continuously sutured with 1-0 vicryl (Figure 9) in two, three or four layers, which depended on the depth of the uterine incision and no dead space was created during the suturing. The pelvic cavity was washed carefully and repeatedly to reduce the adhesions, and sodium hyaluronate covered the uterine surgical defect, which prevented the adhesions.

The final limitation on the development of the laparoscopic adenomyomectomy procedure was the post-operation recurrence. In theory, complete removal of an adenomyoma could reduce the risk of recurrence. In the present study, the authors removed the regional lesions as much as possible. After the surgery, all the patients were treated monthly with GnRHa for three or six months to prevent recurring attacks. GnRHa decreases the expression of aromatase cytochrome P450, which is related to the recurrence of adenomyomas [3]. For the postoperative patients who were not planning for fertility, using LNG-IUD as an adjunct treatment was safe and effective and could decrease recurrence rate.

In the present study, the pregnancy rate was 79.4%
after laparoscopic adenomyomectomy surgeries and the fertility rate was 51.5%. All of the 50 women were delivered by cesarean section, and 44 patients returned to the present hospital for their surgeries. Seven of the patients (15.9%) who returned were found to have severe pelvic adhesions, 22 (50%) patients had mild or moderate adhesions, and 15 (34.1%) patients had no obvious adhesions. The present authors found that a laparoscopic adenomyomectomy could enhance fertility and reduce pelvic adhesions. However, the pregnancy outcomes require long-term follow-up in future studies. This study shows that laparoscopic adenomyomectomy is feasible and safe for severe adenomyosis.

References


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Migraine management in pregnancy

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Summary

In spite of the fact that migraines are one of the major problems seen by primary care providers, almost half of people with migraines do not obtain appropriate diagnosis or treatment. Migraine occurs in about 18% of women, and is often aggravated by hormonal shifts occurring around women's menses, during pregnancy, and during perimenopause. Quality of life with migraines is often greatly diminished, and many women miss work days with migraines. In women, the hormonal fluctuations seen during pregnancy and lactation can affect migraine frequency and magnitude. Understanding the evaluation of headache in pregnancy is important, especially given the increased risk of secondary headache conditions. Pregnancy and lactation can complicate treatment options for women with migraine because of the risk of certain medications to the fetus. This review includes details of the workup and then provides treatment options for migraine during pregnancy and lactation.

Key words: Migraine, pregnancy, headache, treatment.

Introduction

Most epidemiological studies have demonstrated that the majority of women suffering from migraine note remarkable and increasing improvement of their attacks during pregnancy, from the first to the third trimester. Women suffering from migraine are also at higher risk of developing gestational hypertension, preeclampsia, or vascular complications related to pregnancy including ischemic stroke and other vascular events in the peripartum period. Primary headaches are most common in women during their reproductive years and are affected by the hormonal fluctuations during pregnancy. Pregnancy creates alterations in maternal physiology which predispose to unique neurologic disorders. Pre-eclampsia, eclampsia, certain types of ischemic and hemorrhagic stroke, reversible cerebral vasoconstriction syndrome, posterior reversible encephalopathy syndrome, and headache all appear to share a common origin from vascular endothelial dysfunction, with overlapping clinical presentations. Among primary headaches, migraine is the form more sensitive to the ovarian hormonal milieu. Migraine without aura benefits from the hyperestrogenic state of pregnancy and the lack of hormonal fluctuations, while migraine with aura presents distinctive features. Indeed, a very strong improvement of migraine without aura has been documented across gestation, and only a minority of pregnant women still suffers during the third trimester. On the other hand, fewer women with migraine with aura report improvement or remission, and new onset of aura may be observed during pregnancy. After delivery, breastfeeding exerts a protective action on migraine recurrence. The persistence of migraine during gestation seems to affect neonatal outcomes, and several studies indicate a link between migraine and an increased risk of developing gestational hypertension, preeclampsia and other vascular complications. This article reviews the epidemiology, prognosis, and management of primary headaches during pregnancy and lactation, and considers secondary headaches that are important to exclude.

Prevalence

Migraine is one of the most common neurological disorders among women, with a female/male proportion of 3-4:1. Women, compared with men, have a 1-year migraine prevalence nearly three fold higher and lifetime incidence more than twofold higher. Its prevalence among women varies from 4% before puberty to 25% during the reproductive life, decreasing later in perimenopause and postmenopause. Moreover, menarche, menstruation, pregnancy, and menopause as use of oral contraceptives and hormone replacement treatment may influence migraine occurrence. Until puberty, migraine affects both sexes equally. After the menarche there is an increasing prevalence of migraine in women. Changes in migraine frequency can also occur during pregnancy, lactation, and contraceptive use. During pregnancy, the frequency of migraine de-increases in most women, with an increase in its remission from the first to the third trimester. One of the...
Headache types and risk factors

The International Classification of Headache Disorders categorizes primary and secondary headaches [6]. The most frequently reported triggers for tension-type headache and migraine are stress, irregular or inappropriate meals, high intake or withdrawal of coffee and other caffeine-containing drinks, dehydration, sleep disorders, too much or too little sleep, and reduced or excessive physical exercise [7, 8].

Primary headaches

Primary headaches account for the majority of headaches during pregnancy. Of the primary headaches, tension-type headache and migraine generally improve during pregnancy. A common cause of daily headache in a patient with a history of primary headache is medication overuse headache. They do not change in frequency compared to the prepregnancy state. Migraine headaches can occur with or without aura. They are frequently unilateral and pulsatile. Fluctuations in the estrogen level rather than the absolute level may trigger a migraine. The effect of pregnancy on cluster headache is limited because of the rarity of the condition, and the data are conflicting. During pregnancy migraine without aura tend to improve due to the stable level of estrogen. Sances and colleagues found that migraine without aura significantly improved in over 87% of patients by the third trimester. Breast-feeding reduced migraine recurrence within the first month after childbirth to 50% of patients in comparison to 86% who bottle-fed. The benefit lasted for 6 months. One year after delivery, 80% of both populations had experienced recurrence of headache [9].

Recurrent episodic headaches that last between 4 and 72 hours and are associated with photophobia, nausea, and disability in an otherwise well person are typical features of migraine. Up to 60% to 70% of women with preexisting migraine report fewer migraine attacks during pregnancy [10].

Secondary headaches

This type of headaches are caused by other conditions distinct from primary headaches. Secondary causes of headache that are more likely to occur during pregnancy include cerebral venous thrombosis, posterior reversible encephalopathy syndrome resulting from eclampsia, postdural puncture headache, stroke, and pituitary apoplexy. While migraine and tension-type headaches do not have a negative impact upon pregnancy directly, the following differential diagnostic considerations should always be kept in mind. The clinician should inquire about the character of the headache (acute onset? severe?), lack of similar headaches in the past, change in headache pattern and progressively worsening or persistent headache. On examination the clinician should be vigilant for focal neurologic findings, decreased level of consciousness, emesis or syncope at the onset of headache, convulsion, fever and meningismus [11].

Diagnosis

Migraine that occurs for the first time during pregnancy necessitates a detailed history, physical examination and radiographic or laboratory tests as appropriate to exclude secondary causes of headache which may resemble migraine. Migraine is also associated with an increased risk of hypertensive disorders of pregnancy. Specific questions can help to evaluate secondary causes of headache that may need urgent assessment. Because secondary headaches can occur in a patient with a long-standing history of primary headache, it is important to elicit new symptoms. This approach can separate those who need further investigation from those with benign secondary headaches or typical histories of primary headaches. The latter can be reassured, treated, and followed by the neurologist or primary care physician as appropriate. Examination should focus on assessment for signs of concerning diagnoses such as infection or hemorrhage; severe hypertension; and neurologic signs in including papilledema or hemorrhages on fundoscopy, neck stiffness, altered consciousness, or weakness.

Treatment

Headache presenting in pregnancy is of significant concern to the affected woman. Quick and correct diagnosis leads to the optimal management, minimizing risks to the pregnancy. Migraine in pregnancy can cause considerable concern to both patient and doctor, particularly if migraine starts for the first time during pregnancy or if the woman has her first attack with aura. Most headaches follow a benign course during pregnancy, although migraine is associated with increased risk of hypertensive disorders of pregnancy and stroke. Several strategies have been developed to distinguish secondary headaches that need urgent assessment and management from benign primary and secondary headaches and to minimize the risk of misdiagnosis. Primary headaches are common and typically improve during pregnancy. Management of primary headaches during pregnancy is essentially similar to management in the non-pregnant state, with a few exceptions. Secondary causes of headache that are more likely to occur during pregnancy include cerebral venous thrombosis, posterior reversible encephalopathy syndrome resulting from eclampsia, postdural puncture headache, stroke, and pituitary apoplexy. There is often confusion regarding which medicines are
Migraine management in pregnancy

 safe to use during pregnancy and breastfeeding, leaving many women unable to control their attacks effectively.

Pregnant women with tension-type headache or migraine should be encouraged to avoid skipping meals, to take regular exercise, to drink plenty of fluids, and to maintain a regular sleep pattern. Alcohol and smoking are potentially harmful to the fetus and should be avoided during pregnancy.

Nondrug therapies such as:
- Relaxation,
- Biofeedback,
- Physical therapy
- Acupuncture
- Magnesium sulfate
- Vitamin B2, D
- Coenzyme Q10 are safe and may be effective in pregnancy.

Coenzyme Q10 daily is effective for migraine prophylaxis and, when taken during pregnancy, has been associated with a significant reduced risk of preeclampsia.

Medical treatment

Acetaminophen
It is the analgesic of choice for the short-term relief of mild to moderate pain and pyrexia. Recommended dosage of acetaminophen is 4 g or less per day.

Aspirin
While aspirin can be taken during the first and second trimesters of pregnancy, it is best avoided near term because of increased risk of prolonged labor, postpartum hemorrhage, and neonatal bleeding. It should not be taken regularly during breast-feeding because of the theoretical risk of Reye syndrome and impaired platelet function in infants.

NSAID
Ibuprofen is the NSAID of choice during the first or second trimester. NSAIDs and aspirin should be avoided during the third trimester because chronic use or high doses after 30 weeks are associated with an increased risk of premature closure of the ductus arteriosus and oligohydramnios.

Opiates
Although safe for treatment of moderate to severe pain in pregnancy, opiates are inappropriate for migraine because they aggravate nausea and reduce gastric motility

Cluster headache treatment
Preferred treatments for cluster headache during pregnancy and lactation are verapamil or prednisolone. Acute treatment includes oxygen (100% at 7 L/min for 10 to 15 minutes at onset of attack) or subcutaneous or intranasal sumatriptan.

Emergency treatment
During pregnancy and lactation, prochlorperazine 10 mg or chlorpromazine 25 mg to 50 mg by IM injection are effective for emergency headache Relief. IV magnesium sulfate 1 g given intravenously over 15 minutes was well tolerated and effective in acute cases [17]. Corticosteroids successfully treat intractable nausea and vomiting in hyperemesis gravidarum. A 6-day reducing course of prednisolone [18].

Harmful treatment
It should be noted that the some medications used to treat migraine in the nonpregnant patient carry increased risk to the fetus. Valproate may produce neural tube defects due to inhibition of folic acid metabolism. Other medications to avoid include topiramate, lithium, phenobarbital, angiotensin receptor blockers and second-generation ACE inhibitors, atenolol, paroxetine, methysergide, certain nonsteroidal agents and ergot alkaloids in the first trimester [19].

Breastfeeding in migraine
Breast-feeding is encouraged because it maintains the protective effect of pregnancy on migraine headache during the postpartum period. A breast-feeding woman may forego treatment or even stop breast-feeding due to her fears of exposing her infant to medication. It is important to balance the risk of medication exposure with the benefit of migraine treatment. While many medications are considered to be compatible with breast-feeding, studies on breast-feeding women and their infants are rarely done due to obvious ethical concerns. To some extent, most drugs transfer into breast milk. Exceptions include heparin and insulin as their size is too large to cross biological membranes. The transfer of drugs into breast milk is commonly described quantitatively using the milk to plasma concentration ratio. For drugs that appear in breast milk to any significant extent, it may be reasonable to reduce infant exposure by alternating breast and bottle-feeding, or by adjusting the timing of when the medication is taken relative to breast-feeding. This approach may be particularly useful for medications with a short half-life and acute migraine treatments.

Postdural puncture headache
Postdural puncture headache is the most common major complication following neuraxial anesthesia; this adverse
event is particularly frequent in obstetrics. The headache is usually benign and self-limited but if left untreated can lead to more serious complications that may be life-threatening. After accidental dural puncture the most important effective treatment is to leave the catheter inside the dura; epidural morphine infusion may also help. Once symptoms begin, treatment is conservative for the first 24 hours. If this approach fails, the most effective intervention continues to be a blood patch, which should not be delayed beyond 48 hours. If more than two blood patches are required, other possible causes of headache should be ruled out.

Postpartum headache

The large majority of postpartum headaches are recurrences of preexisting primary headache disorders, which recur within a month after delivery in 55% of prior migraineurs. Prior headache history, dural puncture, increasing age, multiparity, and shorter length of second-stage pushing have been identified as potential risk factors for postpartum headache shortly after delivery. Tension-type headaches and migraine flare-ups are the most common headache complications after delivery, but the differential diagnosis of postpartum headache is broad. The clinician must distinguish common headache syndromes from dangerous causes of postpartum headache [21]. Nearly all women report the return of migraine attacks after delivery. Factors accelerating the return of migraine attacks in the postpartum include bottle-feeding and age of 30 years or less. Although preexisting headache often improves during pregnancy, approximately one-third of mothers experience postpartum headache within the first week after delivery making headache one of the three most common reasons for acute care visits during the puerperium.

Menstruel migraine

Migraine is a biological disorder and providing an understanding of the role of estrogen in the frequency and severity of migraine can guide treatment choices. Genomic patterns in adolescent girls differentiate between menstrually related migraine and non-menstrually related migraine. According to some studies, menstruel migraine attacks are accompanied by nausea and vomiting more than non-menstrual attacks although this finding is not unanimously shared by all the studies. Correlations have been identified between premenstrual syndrome and menstrue¿ migraine [22]. Menstrually-related migraine before pregnancy often predicted lack of headache improvement during pregnancy [23]. Management of hormonally influenced migraine involves a clear identification of the relationship between migraine and hormone change. A thorough history and detailed diary are critical in identifying this relationship and in predicting response or following response to hormonal therapies. Although limited, clinical evidence suggests that OCs use in young women with episodic migraine may transform their pattern into chronic migraine. There is a well-documented association between COCs and migraine. They may induce a de novo migraine in women without a previous history of the disease, worsen a previous existing migraine, or change the pattern of a previous existing migraine. Thus, particular attention to changes in migraine patterns following either endogenous or exogenous hormonal changes is crucial. The age at initiation of estrogen replacement therapy appears to be significant with respect to stroke. No increase in stroke occurred in women on low-dose (50 µg or less) transdermal estrogen replacement compared to women not using estrogen replacement. Pharmacologic treatments include acute therapy, with short-term and standard prevention offered where appropriate. Hormonal therapies are not first-line therapies but may be important choices for a woman with migraine whose estrogen fluctuation is continually exacerbating migraine attacks. Overall wellness should also be emphasized; regular exercise, balanced diet, smoking cessation, weight control, and sleep hygiene are important in the management of migraine [24].

Conclusion

Headache is a common symptom in pregnant women. Although most headaches seen in women are primary headache disorders (migraine, tension-type headache), complications or conditions associated with pregnancy can present with a secondary headache. Headaches are common symptoms in idiopathic intracranial hypertension, eclampsia, and reversible cerebral vascular syndrome. Migraines may begin or worsen during pregnancy, but pregnancy tends to reduce migraine frequency and severity. Although it is desirable to avoid medications for headaches during pregnancy, treatment should be considered when headaches are severe and cause significant disability. Being aware of possible treatments for migraine and headaches during pregnancy is essential. Finally, migraine does not adversely affect the outcome of pregnancy in otherwise healthy women. However migraine during pregnancy is associated with increased risk of arterial and venous thrombosis, preeclampsia, and gestational hypertension. Therefore pregnant women with migraine should be monitored for rising systolic or diastolic blood pressure should prompt a check for proteinuria. Breast-feeding is encouraged because it maintains the protective effect of pregnancy on migraine headache during the postpartum period.

References

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Bulking agents – an analysis of 500 cases and review of the literature

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Summary

Introduction: Stress urinary incontinence (SUI) is common, impacts women’s quality of life and generates high costs. Physiotherapy is the first line therapy and if it fails, suburethral slings are currently the gold standard in SUI surgery. Bulking agents injected periurethrally might be a beneficial alternative, but there is a paucity of data on bulking therapy. Aim of the current study was to analyze the efficacy and safety of bulking agents in the setting of a tertiary referral center prospectively. Materials and Methods: In the last 13 years 514 elderly women with SUI were treated by injection therapy with either collagen, hyaluronic acid, ethylene vinyl alcohol or polyacrylamide hydrogel. Subjective and objective outcomes were recorded at the 12 month post-operative appointment using the King’s Health Questionnaire (KHQ) and Visual Analogue Scale (VAS) to describe their incontinence severity, standardized Pad-Test, and urethral pressure profile. Results: Demographic data were equally distributed in all four groups of agents used. Sixty-one patients were lost to follow-up (10.6%). Statistically significant changes were found for maximum urethral closure pressure (MUCP), pad weight, and VAS before and after bulking for the four agents used. Pad-Test was negative in 73.2% of patients after bulking therapy. Subjective assessment showed improvements in general health and role limitations. The overall complication rate was low for all agents. Conclusions: The current study shows an improvement of incontinence after bulking therapy applying subjective and objective outcomes in an elderly population. In contrast to earlier reports, side effects due to injections were few and mild. We can advocate bulking therapy for the treatment of SUI as it is simple, safe, and shows both objective and subjective improvements and relief.

Key words: Stress urinary incontinence; Bulking agents.

Introduction

Involuntary loss of urine during coughing, sneezing, physical exertion or sudden changes of position characterize stress urinary incontinence (SUI) is caused by either sphincter abnormalities and/or urethral hypermobility [1, 2]. SUI with its high socio-economic burden and influence on women’s quality of life is a common problem with approximately 35% of women older than 18 years suffering from involuntary loss of urine, and at the age of 60 it rises to 45% in Europe [3]. Annual costs related to urinary incontinence are estimated to be $27.8 billion in the U.S. [4], and 359 to 655 Euro per patient treated in European countries [5]. Treating incontinence might improve quality of life and cut these costs significantly.

The first step in treatment is pelvic floor rehabilitation [6] followed by surgery if physiotherapy fails. As for surgery, suburethral slings are as effective as colposuspension with lower perioperative morbidity and currently are the gold standard in patients with SUI displaying high and long term cure rates [7, 8]. However, there is a need for alternative therapeutic approaches in patients with significant comorbidities, in women who are unwilling to undergo surgery because of its associated risks, pain and recovery, in patients with current SUI, and in women where surgical options are limited (e.g. post-operatively or after irradiation) [9-14]. The current study focuses on injection therapy with bulking agents as it may be considered as a first-line treatment option in selected patients [11].

Currently, there is a paucity of data comparing bulking agents and inconsistent studies describing the efficacy of bulking agents [10,15]. Hence, the choice of substance still depends on safety considerations, ease of use, availability, and physician preference as there is no strong evidence to one agent being superior to the other [11].

Despite the theoretical advantages of injection therapy, the latest Cochrane review from 2007 concluded that a lack of sufficient data on bulking agents impeded creation of a meta-analysis [10]. The paucity of long term follow-up and health economic data, as well as the finding of a possible placebo effect (improvement in pad weight after saline injections) were further points of criticism in this review [10]. However, another aspect was the lack of a comparison of bulking therapy with physiotherapy [10] which was recently made decrepit as bulking seems to be more effective than pelvic floor training [16]. The Cochrane review con-
cludes that limited data suggest surgery to be objectively superior to bulking, but as patients are equally satisfied with either option and regarding the little side-effects of bulking therapy, it is considered to be a reasonable first-line option [10]. The aim of the current study was to analyze the efficacy and safety of bulking agents in the setting of a tertiary referral center prospectively.

Materials and Methods

Between December 2000 and January 2013, n=514 elderly women with SUI or mixed incontinence were treated by injection therapy with either glutaraldehyde cross-linked bovine collagen, hyaluronic acid/dextranomer copolymer, ethylene vinyl alcohol or polyacrylamide hydrogel in the Women’s Hospital, Chemnitz-Rabenstein, Germany. The choice of bulking agent was dependent on substance availability and patient’s allergy towards collagen.

Demographic data including age, body mass index (BMI), previous incontinence operations, number of injections, and perioperative data were noted. The study was approved by the local ethical committee and all patients gave informed consent to participate in the study.

The King’s Health Questionnaire (KHQ) assesses quality of life and is widely used in patients suffering from incontinence [17]. It is validated in several languages including German [18]. The questionnaire deals with the domains general health perception, role limitation (e.g. household, cleaning, shopping), physical and personal limitation (walking, sports, travel, social life, relationship, sex, family life), emotions (depressed, anxious, nervous, feeling bad about oneself), sleep (feeling worn out, tired), and incontinence impact (pad usage, need to change underwear, restrict drinking, fear of bad smells). Moreover, bladder problems are specified in the KHQ as questions for frequency symptoms, nocturia, urgency, stress incontinence episodes, coital incontinence, urinary tract infections, and bladder pain exist. The scores for each domain range from 0 to 5 and 1 to 5, respectively, are added up and a change of at least five points is considered significant [18].

Subjective outcome was further assessed with the patients judging their incontinence severity on a visual analogue scale (VAS). The VAS is a validated tool to assess health and satisfaction in patients, to investigate pain and for measuring attitudinal attributes and quality of life [19]. Additionally, as objective measurements a standardized two-hour in-office Pad-Test according to International Continence Society (ICS) recommendations [20] was performed and residual urine was measured using transabdominal ultrasound. Additionally urethral pressure profile was measured using microtip catheters. Microtip measurements were taken in the 45° upright position with the patient at rest and at bladder capacity using a 8 Fr double microtip transducer withdrawn at one mm/sec and the transducer was orientated in the three o’clock position with one transducer inside the bladder and the second one distally positioned in the urethra. Three consecutive measurements were taken for each patient and the average was calculated.

Before intervention and afterwards, urinary tract infections were excluded using dipstick screening and infections or bacteriuria was treated. For the injection procedure, the women were placed in the lithotomy position, 10-20 ml of 1% lidocaine were injected in the periurethral tissue at four and eight o’clock and the bulking agent was injected transurethrally into the submucosa under cystoscopic control. Two to three deposits were placed in the mid-urethra and quantity was decided by the surgeons judgment of coaptation. The needle position was corrected if it was suspected to not be in the mucosa or if there was extravasation of the bulking agent. If coaptation was considered appropriate, the bladder was emptied. Patients received a single-shot antibiotic prophylaxis with trimethoprim-sulfamethoxazole and were discharged if post-micturition residual volume was < 100 ml. Evaluation of the patients was performed 12 months postoperatively. All adverse events were monitored and registered. If the operation was not successful, the women were offered a further injection after six weeks. For statistical analysis, Graph Pad Prism version 5.0 was used to calculate Student’s t-Test and Mann-Whitney Rank Sum Test.
Results

The four types of bulking agents used in this study were collagen (n=312), ethylene vinyl alcohol (n=104), hyaluronic acid (n=54), and polyacrylamide hydrogel (n=44) resulting in a total number of 514 patients. Demographic data were equally distributed in all four groups: age (median 79 years, range 41 to 91), BMI (median 29 kg/m², range 21 to 41 for polyacrylamide hydrogel, and 19 to 41 for the other agents, respectively), previous incontinence operations (median 1, range 0 to 4), number of injections (median 1, range 0 to 4), hospital stay (median 2 days, range 1 to 3 except for one maximum stay of 34 days in a patient where complications occurred after a collagen injection), and operation time (median ten minutes, range 10 to 25). Eighty percent of patients answered in German, 18% in French, and 2% in English. Sixty-seven percent of the patients suffered from SUI and 33% from mixed urinary incontinence (MUI). Despite one-third of the patients suffering from MUI, the complaint of SUI was predominant. Sixty-one patients were lost to follow-up.

For the agents used, the median changes in maximum urethral closure pressure (MUCP) and Pad-Test are shown in Figure 1. VAS score as a measurement of self-reported disturbance is illustrated in Figure 2.

Analysis with the Mann-Whitney Rank Sum Test for not normally distributed groups showed statistically significant changes for MUCP, Pad-weight and VAS, before and after bulking for all four agents used (all \( p < 0.001 \) except for MUCP with hyaluronic acid \( (p = 0.004) \) and for polyacrylamide hydrogel \( (p = 0.011) \)). Estimating that a Pad-Test is negative equal or below two grams, the exact percentage of objective success is 73.2% of the patients.

In the subjective assessment of the patient’s quality of life after bulking therapy, the domains’ general health and role limitations of the KHQ were rated significantly better (Figure 3) while the other domains showed at least no deterioration of quality of life aspects.

The overall complication rate was low for all agents (collagen 3.2%, ethylene vinyl alcohol 5.7%, hyaluronic acid 5.6%, and polyacrylamide hydrogel 0%). The most serious side effects were found for collagen with two women having a late-onset allergic reaction to collagen at three and six weeks post-operatively, respectively, requiring analgetics and steroids. One of these women had to be hospitalized for 34 days. Another serious event \((n=1)\) was material exposure of ethylene vinyl alcohol after two years requiring cystoscopic removal of the agent resulting in an incontinence relapse. Further complications: urinary retention for one to seven days treated with intermittent catheterization using a self-lubricating catheter and ultrasound check of residual urine after next micturition (collagen, \( n=4 \)); simple urinary tract infection treated with antibiotics (collagen, \( n=4 \) and ethylene vinyl alcohol, \( n=3 \)); temporary frequency requiring anticholinergics for up to two weeks (ethylene vinyl alcohol, \( n=1 \) and hyaluronic acid, \( n=1 \)); worsening of incontinence (hyaluronic acid, \( n=2 \)); tachyarrhythmia during local anaesthetic injection (before ethylene vinyl alcohol procedure, \( n=1 \)); blood stained urine for three days (ethylene vinyl alcohol, \( n=1 \)).
Discussion

The current study shows an improvement of incontinence after bulking therapy applying subjective and objective outcomes in an elderly population. Side effects due to injections were few and mild. The 514 women in this study showed a similar demographic distribution for each of the four bulking agents used. Thus, comparison of the results is not biased by different patient collectives.

Outcomes after bulking therapy for four different bulking agents are studied. Although ethylene vinyl alcohol and hyaluronic acid have been abandoned because of safety issues [15, 21, 22] their outcomes are in line with the other agents and data are helpful in the evaluation of the bulking principle.

Two types of outcomes are distinguished in this study: objective measures (MUCP, Pad-Test) and subjective assessment (VAS and KHQ). The results for the objective measurements were clear cut and showed a significant improvement. MUCP might reflect the anatomic improvement with a better coaptation of the urethral mucosa and the Pad-Test indicates the decrease of urinary loss.

The subjective assessment revealed statistically significant improvements on the VAS and several domains of the KHQ, namely general health and role limitations. The other domains were equally or higher valued post-operatively yet not significantly. The KHQ especially deals with the questions in how far women still use incontinence pads and fear bad smell. Despite the incontinence being improved or cured, patients might fear urinary leakage and pad use in everyday life. This might explain why incontinence impact in the KHQ did not improve. Patient reported outcomes in incontinence therapy are important as objective parameters to verify improvement of urine leakage and might be necessary to compare interventions, but the impact on quality of life may differ from objective measurements substantially [14]. Achievement of what is best for our patients by investigating and discussing treatment goals [11] is possible only if we have a sound knowledge of subjective perceptions of a therapy’s consequences.

The most recent Cochrane Review on bulking therapy stated an unsatisfactory basis for practice and injection therapy was considered useful as an option for short-term symptomatic relief in selected patients with comorbidities [10]. Nevertheless, the minimal invasiveness, favourable safety profile, high cure rates at least in short term, and improvement of quality of life support the appliance of bulking therapy [11,14]. Moreover, a prior bulking therapy seems to not negatively affect outcomes if future anti-incontinence surgery is needed [23] and vice-versa bulking can be used after failed mid-urethral sling placement with a low cure rate, but high patient satisfaction with no significant complications [24].

The efficacy of the bulking principle in general is not yet proven [25]. Continence amongst others is achieved by urethral mucosal coaptation established by the mucosa itself, sub-mucosal vascular cushions, and smooth muscle activ-
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ity [10]. Injection therapy into the urethral sub-mucosa creates cushions and is therefore meant to improve coaptation [10]. Additionally, bulking agents are suggested to act as a central filler volume which lengthens the muscle fibers and thus increases urethral sphincter strength [26]. However, urodynamic data are limited and according to the Cochrane review, urodynamic measures should be included in trials if the mechanism of any action is to be verified [10]. In this regard, the present data including MUCP measurements follow these recommendations and the subjective outcomes argue for efficacy of the bulking principle.

Although urethral bulking was thought to be particularly helpful in women with a low MUCP (intrinsic sphincter deficiency, ISD) [27], bulking is equally effective in both urethral hypermobility and intrinsic sphincter deficiency [11, 14]. An endoscopic delivery of the bulking agents under local anesthesia is typical, yet a blind administration via special devices may be considered as beneficial [28]. The adequate site for injection is the mid-urethra [29] and the mode of delivery of the agent (periurethral vs. transurethral) leads to similar outcomes but increased early complications if administered periurethral [10]. Two or three injections are likely to be required to achieve a satisfactory result [10]. A learning curve for mastering injection therapy via an endoscope seems to be present [15]. Data on cost-effectiveness of bulking are inconsistent with being cheaper than tension-free vaginal tape (TVT) at least in the short term, while economic modelling predicts a higher cost for injection therapy [11].

Poor long-term results and the necessity of repeat injections hamper the use of bulking agents and factors that impact treatment success and durability are to be identified [30]. The search for the ideal bulking agent aims at improving the bulking procedure. The properties of an ideal bulking agent should be durable, biocompatible, hypoallergenic, deformable, non-immunogenic, leading to minimal inflammatory, and fibrotic response, and these particles - usually suspended in a bio-degradable carrier gel - should be large enough to prevent migration (>110 µm) [10, 14, 27]. To date, this does not exist. Wide confidence intervals and a diversion of outcome parameters complicate comparison of agents in earlier studies [31]. In the present study, follow-up beyond 12 months was unfeasible as most patients were referred for the bulking procedure only and after the one-year control, patients were followed-up by their referring doctors. Thirty percent of patients (only including the ones who were followed-up by us or re-referred) needed further injection therapy after 12-18 months. Nevertheless due to this geriatric age group, the rate for re-injection might be even higher: patients may become seriously ill and unable to turn in the incontinence clinic or may even decease before incontinence reoccurs.

Silicone particles, calcium hydroxyapatite, ethylene vinyl alcohol, carbon-coated zirconium beads, porcine dermal implant, and glutaraldehyde cross-linked bovine collagen show equal improvements [10, 32-34], with variations in long and short term outcomes [34-36]. Cure or improvement rates vary between 62% and 80% or 20% and 86%, depending on the source used [9, 10, 37]. Autologous fat proved to be unsafe (one death due to fat embolism) and a favourable outcome was not found [10]. Polytetrafluorethylene made from teflon has been abandoned from clinical use because of particle migration [10]. Paraffin, ethylene vinyl alcohol, and hyaluronic acid have been abandoned because of safety issues [15, 21, 22]. Polyacrylamide hydrogel was specifically developed for urethral bulking being biocompatible, non-biodegradable, non-allergenic, non-migrational, atoxic, stable, and sterile [9, 15]. Its efficacy is proven and its properties might circumvent drawbacks of other agents mentioned [15, 38]. Further experimental agents are evaluated [10, 14, 39-41]. However, as in the present study, a large number of patients showed similar outcomes for all four different agents used and the usefulness of bulking therapy regardless of the specific agent was demonstrated.

Bulk agents have become popular with a substantial efficacy and low morbidity but complications are not to be neglected [14]. Although urethral bulking is considered to be safe and simple [9, 14, 31], there are several reports on complications caused by the different bulking agents like urethral erosion [15], urethral prolapse [42], urethral diverticuli [43], periurethral pseudocyst and mass formation [44], retention, de novo frequency, sterile and non-sterile abscess formation [45], hypersensitivity and urinary infection [15, 43], granuloma formation, and possibly carcinogenesis due to particle migration [43], need for endoscopic evacuation due to bladder outlet obstruction [46]. Treatment-related (minor) adverse events were recently found to occur in a range of 22 to 50% with urinary tract infections being the most common one [15, 38]. The side effects noted in the present 514 patients are not in line with these data as the authors had very low and almost only minor side effects related to bulking therapy.

The large number of patients is the major strength of the present study. Another advantage is the assessment of both subjective and objective outcomes as the subjective outcome might reflect the patient’s goals more accurately. The use of validated tools underlines these findings.

A weakness of this paper is the use of four different types of bulking agents; however, this was entirely due to availability of substances and probably reflects the “real world”, with bulking agents appearing and disappearing on the market. A further weakness is the patients who were lost to follow-up (10.6%). The present authors do not know why these patients were lost to follow-up. It might be due to dissatisfaction and if they count these patients as still incontinent, hence the success rate of bulking might be lower.

In conclusion, the authors can advocate bulking therapy for treatment of SUI as it is simple, safe, and shows both objective and subjective improvements and relief in women.
although it is less effective than slings [47]. This study might help support the use of bulking agents because of efficacy and minimal invasiveness.

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First trimester maternal serum PAPP-A levels and associated pregnancy complications in intrahepatic cholestasis of pregnancy

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Summary

Purpose: To investigate first trimester maternal serum pregnancy associated plasma protein A (PAPP-A) multiple of the median (MoM) in cases with intrahepatic cholestasis of pregnancy (ICP). Obstetric complications and relation with PAPP-A MoM were also evaluated. Materials and Methods: This was a retrospective case-control study. After exclusions, for each ICP case, two controls with uncomplicated singleton pregnancies were randomly selected. PAPP-A MoM of ICP cases with and without obstetric complications, and the control group were compared with each other. Results: Total incidence of ICP was 0.99% (138/13988). The study included 113 singleton pregnant women. Rates of gestational diabetes mellitus (GDM), preeclampsia (PE), fetal growth restriction (FGR), preterm labor (PTL), and hypothyroidism in cases with ICP were 21.2%, 7.9%, 10.6%, 18.6%, and 5.3%, respectively. Median PAPP-A MoM were 0.93 in ICP group and 1.10 in control group (p > 0.05). PAPP-A MoM levels were not significantly different either between the ICP group with complicated pregnancies and the control group or between the ICP group without complicated pregnancies and the control group (p > 0.05). Conclusion: ICP incidence was similar to other European countries. Rates of obstetric complications especially GDM were higher than expected in general pregnant population. ICP is not considered as pregnancy complications that have low PAPP-A MoM levels.

Key words: Intrahepatic cholestasis of pregnancy; PAPP-A; Adverse pregnancy outcome.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is primarily a liver disorder, which usually presents in the third trimester of pregnancy. It is characterized by maternal pruritus, elevated liver enzymes, and total bile acid (TBA) levels in the absence of any other skin or liver disease. The sign and symptoms completely resolve after delivery. Although environmental, genetic, and hormonal factors are believed to be responsible from this disorder and the etiology and pathogenesis are not exactly understood [1, 2]. ICP is associated with abnormal biliary transport across the canalicular membrane [3]. Several mutations have been identified in some of these patients, which might lead to dysfunction of bile salt transport proteins. Mutations in ABCB4 gene (ATP-binding cassette B4) coding for the phospholipid transport protein MDR-3 (multidrug resistance protein 3) and ABCB11 gene (ATP-binding cassette B11) coding for the bile acid transport protein bile salt export pump (BSEP) are the most well defined ones [4].

The ICP incidence in Europe ranges from 0.1% to 1.5% of pregnancies and wide geographical variations are observed throughout the world [5-7]. Maternal prognosis is usually good, but it is associated with some adverse obstetric outcomes, including preterm birth (PTB), meconium staining amniotic fluid, fetal distress, and even stillbirths. Although stillbirth rates were initially reported to be as high as 15%, recent evidence indicates that it is about 3.5% [8]. Antenatal care might be important in improving fetal outcomes, whereas some of the reports are not in accordance with this fact [9, 10]. For the treatment of ICP, ursodeoxycholic acid (UDCA) provides best response in relieving pruritus and reduction of maternal bile acid and liver enzyme levels, and additionally it may have a role in preventing perinatal complications [11, 12]. Induction of labor at 37 to 38 weeks of gestation is generally recommended [9].

Recent reports indicate an increased risk for gestational diabetes mellitus (GDM) as well as preeclampsia (PE) in pregnant women with ICP [12, 13]. Long term risks might include hepatobiliary diseases [14].

Maternal serum biochemical markers used in fetal aneuploidy screening such as pregnancy associated plasma protein A (PAPP-A) human chorionic gonadotropin (hCG), and alfa-fetoprotein (AFP) have been extensively investigated in obstetric risk assessments [15]. Low 11-14 week PAPP-A values are known to predict adverse perinatal complica-
tions like PE, PTB, and small for gestational age infants, as well as GDM [16, 17]. PAPP-A levels were also investigated in early pregnancy complications such as hyperemesis gravidarum and threatened abortion [18, 19].

PAPP-A is a protease for insulin like growth factor binding protein-4 (IGFBP-4). As it increases the breakdown of IGFBP-4, free insulin like growth factor (IGF) concentration is increased [20]. IGF is important in regulating trophoblast invasion of the decidual cells. Thus, low PAPP-A levels are associated with decreased free IGF concentrations, leading to impaired trophoblastic invasion, poor placental perfusion, and pregnancy complications such as PE, fetal growth restriction (FGR), and preterm labor (PTL), as shown in many studies [21].

In a study performed by Muravska et al., patients with ICP (n=15) had increased serum levels of PAPP-A compared to controls [22]. As far as the present authors know, there is no published data with a larger study population, thus the relation of PAPP-A and ICP is unclear. The present authors’ aim to perform this study was to determine the incidence of ICP in their pregnant population, and to investigate the first trimester maternal serum PAPP-A levels in their ICP cases, dividing them further into two groups with and without any pregnancy complications.

Materials and Methods

This retrospective case-control study was conducted at Başkent University School of Medicine, Department of Obstetrics and Gynecology. The study group included patients with the diagnosis of ICP between the years 2007 and 2013 inclusive, in Ankara, Istanbul, and Adana hospitals. The following criteria were required for the diagnosis of ICP: pruritus and elevated fasting serum TBA (>ten µmol/L) with exclusion of any other liver or skin disease.

Antenatal records of the cases were evaluated and maternal age, gravidity, parity, clinical symptoms, liver enzymes (ALT, AST), and TBA levels, gestational age at diagnosis, gestational age at delivery, delivery route, birthweight, sex of the newborn, associated pregnancy complications such as GDM, PE, FGR, preterm premature rupture of the membranes (PPROM), and PTL as well as medical problems such as hypothyroidism, were noted. Normotative data for fetal growth based on birthweight was used for diagnosis of FGR with the cut-off of 10th percentile.

Multiple pregnancies, pregnancies with fetal chromosomal or structural anomalies, and those whose deliveries did not take place in Başkent University Hospitals were excluded from the study. After exclusions, for each case with ICP, two maternal age, gravidity and parity matched controls were randomly selected among singleton deliveries without any pregnancy complications.

PAPP-A values of the cases and the controls were obtained from routine first trimester aneuploidy screening tests performed in the present hospitals between 11-14 weeks of the pregnancy. An immunoanalyzer with PAPP-A kits was used to measure maternal serum levels of PAPP-A. Gestational age was determined according to the fetal crown-rump length (CRL) measured on the day of serum sampling. Maternal serum PAPP-A levels were adjusted for ethnicity and body mass index (BMI), and then expressed as multiples of the gestational age specific median (MoM), by using the prenatal screening program PRISCA 4.0.

Statistical analyses were performed using SPSS program. Parameters were compared between groups by using Mann Whitney U test. Chi-square test was used for the statistical analyses of the cross-tables. Results were accepted to be significant when *p value was < 0.05.

Results

There were 143 patients diagnosed with ICP during the years 2007 and 2013 inclusive in Başkent University Ankara, Istanbul, and Adana hospitals. Among those, 116 were singleton and 27 were multiple pregnancies. Deliveries of three singleton and two multiple pregnancies were not in Başkent University Hospitals. The incidence of ICP among the deliveries performed in the present hospitals during the years 2007 and 2013 inclusive was 0.99% (138/13988). This value was 0.86% for singletons (112/13186) and 3.12% for multiples (25/802). Out of 138 ICP cases whose deliveries took place in the present hospitals, 38 were assisted reproduction pregnancies including 21 multiple pregnancies.

Multiple pregnancies and those whose deliveries that did not take place in Başkent University Hospitals were excluded from the study group with ICP. There were no pregnancies with fetal chromosomal or structural anomalies. The control group of these 113 cases with ICP included 226 pregnancies randomly selected among singleton deliveries without any pregnancy complications.

The study included 64.6% cases that were nulliparous (73/113). The mean maternal age was 31.26 ± 5.44 years ranging between 20 and 53 years. The median gestational age at diagnosis of ICP was 33 weeks, ranging between 13 and 40 weeks. The mean serum TBA level at diagnosis was 35.06 ± 45.57 µmol/L; it ranged between 10.2 and 366 µmol/L. Excluding one case who aborted at the 15th gestational week, n=112.

The rates of associated obstetric complications and medical problems in cases with ICP were as follows: GDM – 24/113 (21.2%), PE – 9/113 (7.9%), FGR – 12/113 (10.6%),
PPROM – 2/113 (1.8%), PTL – 21/113 (18.6%), and hypothyroidism – 6/113 (5.3%). Meconium stained amniotic fluid was observed in four cases (4/113, 3.5%). Stillbirth was observed in two cases (2/113, 1.8%). Large for gestational age (LGA) newborn was observed in four cases (4/113, 3.5%). UDCA was used in 56/113 cases (49.6%).

Out of 113 study cases with ICP, 53 had first trimester PAPP-A MoM levels; therefore those levels were compared to the levels of 106 control women matched for those 53 cases. Median PAPP-A MoM levels were 0.93 and 1.10, respectively. There was no statistically significant difference between the two groups (* Mann Whitney U Test. NS: nonsignificant.

There are several studies in the literature showing that when there is no fetal chromosomal abnormality, low PAPP-A levels observed in the first trimester, aneuploidy screening might predict a high risk for adverse pregnancy outcome including PE, FGR, PTB, and GDM [17, 23]. Muravskas et al., have investigated the relation of maternal serum PAPP-A levels with PE, threatening PTL, FGR, and ICP in a total of 165 women. Patients with ICP (n=15) had increased serum levels of PAPP-A compared to controls [22]. In the present cases with ICP (n=53), first trimester maternal serum PAPP-A levels were not significantly different from those of uncomplicated control pregnancies (n=106). Even in ICP cases with obstetric complications (n=20), either GDM, PE, FGR, PPROM or PTL, which are known to be associated with low first trimester PAPP-A levels, median PAPP-A level was 0.83 MoM, not significantly different from that of the ICP group without any obstetric complications (0.99 MoM) (p > 0.05) or than that of the control group (1.10 MoM) (p > 0.05). Therefore ICP is not considered as pregnancy complications which have low PAPP-A MoM levels. The results may be more accurate in larger study groups.

While the exact cause of ICP is not known, abnormal biliary transport across the canicular membrane is thought to be the major defect in this process. High estrogen levels may play additional role and inhibit the sulfation and the transport of bile acids by exerting effects upon NTCP (Na-taurocholate co-transporting polypeptide) and BSEP [24]. As estrogen concentrations markedly increase with advancing pregnancy, the abnormality in the transport mechanism becomes more obvious and the clinical symptoms as well as the laboratory findings, that is, increased maternal serum TBA, liver enzyme and even bilirubin levels, are observed. Although the underlying pathophysiologic mechanism is probably there initiating from the beginning of the
pregnancy, ICP as a disease, becomes apparent in late gestation.

Impaired bile acid metabolism and transport across the placenta leads to increased TBA accumulation in the fetal circulation similar to which occurs in the maternal circulation. Elevated maternal TBA levels affect placental hormone production, chorionic vessel constriction, placental transport, and increase myometrial sensitivity to oxytocin [25, 26]. The defect in the bile acid transport system most probably is present even in early pregnancy, either because of genetic mutations or environmental factors or hormonal effects especially those of increasing estrogens. Gradually accumulating bile acids in placental and fetal compartment might have an adverse effect on the trophoblastic invasion process leading to poor placental development. Indeed, pathological examinations of the placental tissue in ICP have revealed increased terminal villous surface area and capillary vessels together with increased syncytial knots indicating chronic hypoxia [27]. This may be the most logical explanation for the increased risks of obstetric complications observed in women with ICP, such as PE, FGR, and PTB. Although it is expected that these complicated pregnancies should have significantly lower first trimester PAPP-A MoM levels, the present study results did not indicate to be so. The reason may be an increased inflammatory reaction in the placental compartment due to gradually accumulating toxic bile acids. Increased proinflammatory cytokines like TNF-α and interleukin-1β (IL-1β), in turn, might stimulate PAPP-A gene expression within the trophoblasts. Thus first trimester PAPP-A MoM levels in ICP may not be as low as expected even in those cases with adverse obstetric outcomes. This fact may also explain the increased PAPP-A levels in ICP observed in the study of Muravská et al. [22]. Of course further investigations are necessary to see whether toxic bile acids begin to accumulate in the placental compartment in the first trimester or not in ICP cases.

Recent studies on ICP indicate an increased risk for GDM and PE in these patients [12,13]. In one report evaluating the ICP cases, the incidences of GDM and PE were found to be 7.2% and 5.1%, respectively [12]. The study of Shemer et al., including 5,477 cases within a 12-year period revealed that the incidence of GDM was 1.3% in ICP compared to 0.4% in the control group. These values were 1.1% and 0.6% for PE. Both differences were statistically significant [6]. In the present study GDM was present in 21.2% of 113 ICP cases. PE was present in 7.9% of the cases. The incidence of GDM observed in ICP patients in the present study was quite high compared to the 3-5% rate expected in general pregnant population [12, 13]. The incidence of PE was in accordance with the expected rate, that is 2-8% [12, 13]. Additionally, in the present study group composed of 113 ICP cases, the rates of FGR (10.6%), PTL (18.6%), and stillbirth (1.8%) were higher than the expected rates of 3-7%, up to 12% and 0.5-0.6% [28, 29] respectively. Previous reports have also revealed increased risk for FGR, PTL, and birth as well as stillbirth in ICP [6, 7, 12]. On the other hand, in the present ICP cases, the rates of PPROM (1.8%) and hypothyroidism (5.3%) were similar to those observed in general pregnant population. LGA, in a recent study, was observed in 4.48% of the pregnancies [30]. LGA rate we have observed in ICP (3.5%) may be considered to be similar to that rate.

ICP incidence varies significantly with geographic and ethnic variations. It is reported to be as high as 15% in Chile and Bolivia and less than 1% in Europe [7,8]. Advanced maternal age, a history of ICP or hepatobiliary disease and multiple pregnancies increase the risk for ICP [8]. To the present authors’ knowledge, this study is the first to report the incidence of ICP among 13,988 deliveries from three regions of Turkey: Ankara- Central Anatolia Region, Istanbul- Marmara Region, and Adana- Mediterranean Region. The incidence of ICP in the present study, that is 0.99%, is in accordance with the figures observed in European countries [7].

References

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Abdominal wall endometriosis occurring after cesarean section: an underestimated complication

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Summary
The aim of the study was to review patients characteristics, describe the exact anatomic locations and size of the endometriosis in the abdominal wall, and discuss the factors that may contribute to mesh use during abdominal wall endometriosis (AWE) resection. Materials and Methods: Patients diagnosed with AWE in their surgical scars from January 2008 to December 2014 were documented. Descriptive data was collected and analyzed. Results: A total of 95 patients with an age ranging from 26 to 48 years, with a mean age of 33.5 ±0.0 years at the time of excision were analyzed. The mean diameter of the mass was 3.25 cm in the present series with an average of 4.97 cm in the mesh group by ultrasound. A total of 18 patients had mesh therapy for fascia defect compared with 77 non-mesh therapy patients. The size of the lesions, the mean duration of symptoms for painful mass, and level of the serum CA125 were statistically different between mesh group and non-mesh group (p < 0.05). Cases of endometriosis lesions limited to the adipose layer had significant lower chance of using mesh (p < 0.05). However, adipose layer endometriosis lesions that had penetrated through the fascia layer and invaded into rectus abdominis muscle layer with/without peritoneum layer had significant higher chance of using mesh (p < 0.05). Conclusions: The more common position for scar endometriosis may be in the adipose layer at the corner of the surgical scar. Mesh therapy should be considered before surgery when the diameter of the abdominal wall mass detected by ultrasound is more than five cm and/or when the lesions invade into rectus abdominis muscle with/without peritoneum tissues from adipose and fascia layers.

Key words: Abdominal wall endometriosis; Surgical scar; Mesh; CA 125.

Introduction
Endometriosis is defined as functional endometrial glands and stroma occurring outside the uterus. The incidence of endometriosis in the abdominal wall is reported to be as high as 3.5% [1]. Many studies [2,3] have addressed the topic of abdominal wall endometriosis (AWE) since Nora et al. first reported 19 cases of AWE associated with cesarean section scars in 1956 [4]. In this retrospective study, the authors describe the exact anatomic locations of endometriosis in the abdominal wall, analyzing and discussing the difference between mesh and non-mesh groups, factors that may contribute to the AWE in the cesarean section surgical scars, and management of patients with AWE.

Materials and Methods
Following approval by the Anhui Medical University Review Board, a retrospective review was performed. Information of patients with AWE from January 2008 to December 2014 was searched from two large teaching hospitals (the first and second affiliated hospitals of Anhui Medical University) with a specific interest in gynecology. All patients had serum CA125 blood test and ultrasound with color Doppler imaging. The largest diameter of the lesion in the abdominal wall was determined by ultrasound examination. All masses proved to be scar endometriosis by pathology after wide excision of the lesion under spinal anesthesia or general anesthesia with mask. The medical records of the patients including parity, age, time-gap between last surgery, onset of symptoms, previous surgeries, ultrasound examination, site of the AWE, initial diagnosis, cesarean section technique, definitive operation, complications, pathology reports, and recurrences were analyzed in detail.

Continuous variable are expressed as mean ± standard deviation (SD). Chi-squared test was performed to compare incidences for each dichotomous variable. Statistical analysis was performed with SPSS 16.0. Difference was considered to be statistically significant when p < 0.05.

Results
A total of 95 patients with an age ranging from 26 to 48 years with a mean age of 33.5 ± 5.0 years at the time of excision were analyzed in this study (Table 1). During the same time from January 2008 to December 2014 in the two teaching hospitals, 16,972 cesarean sections had been performed. The occurrence rate of scar AWE in the study was 0.56%. Ninety patients (94.7%) had a history of one prior cesarean section and five patients (4.3%) had two prior cesarean sections. Seven patients also had a history of endometriosis ovary cystorectomy and another two patients had undergone a hysterectomy by laparotomy because of...
uterine myomas. Eight-five patients (89.5%) had a gradually growing painful nodular abdominal mass in or adjacent to their cesarean incision scars, either non-cyclical \((n=18, 21.2\%\) or cyclical \((n=67, 78.8\%\) in nature. Six patients took analgesic drugs for the relief of severe pain. Only ten patients (10.5%) presented with a mass without pain.

Hard and fixed masses could be palpated near or in the scar position (Figure 1a). However the boundary of the mass was not clear in most of the cases, especially for the small nodules. All the patients underwent ultrasound with color Doppler imaging. The size of the abdominal wall masses varied from 0.5 to 7.0 cm, with an average of 3.25 cm confirmed by ultrasound. Most of the lesions near or in the scars were hypoechoic, vascular, and solid on ultrasound. Ten patients had undergone investigation by MRI due to atypical image in ultrasound. No additional diagnostic procedures such as fine needle aspiration cytology of the lesion under ultrasound guidance or core biopsy were performed in any case. Frozen section during operation was helpful in choosing the correct surgical procedure and no malignant tumor was proved by pathology during or after surgery.

Table 1. — Characteristic data of the patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n=95 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternity</td>
<td></td>
</tr>
<tr>
<td>- Primipara</td>
<td>89 (93.4%)</td>
</tr>
<tr>
<td>- Multipara</td>
<td>6 (6.6%)</td>
</tr>
<tr>
<td>CA125 (U/ml)</td>
<td></td>
</tr>
<tr>
<td>- Normal (&lt;35 U/ml)</td>
<td>67 (70.5%)</td>
</tr>
<tr>
<td>- Elevated (ranged from 36.1~142.9 U/ml)</td>
<td>28 (29.5%)</td>
</tr>
<tr>
<td>Extent of lesions*, n=110 (%)</td>
<td></td>
</tr>
<tr>
<td>- Invaded the adipose layer</td>
<td>102 (92.7%)</td>
</tr>
<tr>
<td>- Invaded the fascia layer</td>
<td>63 (57.3%)</td>
</tr>
<tr>
<td>- Invaded the rectus abdominis muscle layer</td>
<td>32 (29.1%)</td>
</tr>
<tr>
<td>- Invaded the peritoneum layer</td>
<td>13 (11.8%)</td>
</tr>
<tr>
<td>The time-gap, mean±SD (month)</td>
<td></td>
</tr>
<tr>
<td>- Mean duration of symptoms, (ranged from 2~120 months)</td>
<td>28.01±25.21</td>
</tr>
<tr>
<td>- Cesarean section performed and the onset symptoms, (range 2~168 months)</td>
<td>32.76±29.18</td>
</tr>
<tr>
<td>- Previous cesarean section and the time of excision of scar endometriosis, (range 12~180 months)</td>
<td>60.99±36.10</td>
</tr>
</tbody>
</table>

* There are totally 110 abdominal endometriosis lesions among the 95 patients in this study.

Figure 1. — A 36-year-old woman with an abdominal wall endometriosis mass after cesarean section.

a) The skin with a transverse scar where endometriosis mass is located, indicated by a black arrow, is slightly elevated accompanied by pigmentation.
b) Scar endometrioma invading into adipose, fascia, and part of rectus abdominis muscle which is excised with 1.0 cm of the normal tissue around the lesion.
c) The incomplete fascia is difficult to close.
d) Polypropylene mesh is fixed to the fascia by interrupted suture using non-absorbable thread.
e) Haematoxylin and Eosin stained section of the abdominal wall endometrioma with endometrial glands.
f) The incision recovered well at six-week follow-up.
Abdominal wall endometriosis occurring after cesarean section: an underestimated complication

the surgery even for one large mass reaching seven cm in diameter. One patient presented with cyclic cyanotic changes in the subcutaneous tissue adjacent to the cesarean incision scars (Figure 2). AWE lesion extending from the subcutaneous tissue to anterior wall of the uterus had been detected by ultrasound (Figure 3). The patient had both abdominal wall mass resection and uterus repairing procedure in one operation.

Following the imaging procedures, all patients underwent surgery. The surgery performed with/without mesh varied according to the site and size of the scar endometriosis (Table 2). Eighty-two patients complained of one mass (86.3%) in the abdominal wall, 11 patients (11.6%) each presented two lesions in the abdominal wall including ten patients with Pfannenstiel incisions, and one patient with midline incision, and two patients (2.1%) had three painful tubers in the abdominal wall, including one patient with Pfannenstiel incision and one patient with midline incision. Sixty-six patients (69.5%) with Pfannenstiel incisions had 78 lesions and 29 patients (30.5%) with midline incisions had 32 lesions after cesarean. For patients with Pfannenstiel incisions, the masses were found at the left, middle, and right of the cesarean incision scars were 31 (39.7%), 13 (16.7%), and 34 (43.6%), respectively, while in patients with midline incisions, the masses found at the upper, middle, and inferior portions of the cesarean incision scars were 12 (37.5%), seven (21.9%), and 13 (40.6%), respectively.

According to the position of the scar mass, about 1.0 cm of the normal tissue around the lesion was excised (Figure 1b). Polypropylene mesh was be used if the incomplete fascia was difficult to close (Figures 1c, 1d). No special anti-adhesion mesh was required due to peritoneum defects in the study. A total of 18 patients had mesh therapy for fascia defect compared with 77 non-mesh therapy patients. The size of the lesions, mean duration of symptoms for painful mass, and level of the serum CA125 excluding seven patients with a history of endometriosis ovary cys-
Table 2. — Clinical data between mesh and non-mesh use cases.

<table>
<thead>
<tr>
<th></th>
<th>Non-mesh use case</th>
<th>Mesh use case</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD (years)</td>
<td>32.93±5.03</td>
<td>34.88±4.55</td>
<td>0.09</td>
</tr>
<tr>
<td>Size of the lesion, mean ± SD (cm)</td>
<td>2.60±1.10</td>
<td>4.97±1.09</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean duration of symptoms of painful mass, mean ± SD (months)</td>
<td>16.08±14.59 (n=70)</td>
<td>24.00±13.67 (n=15)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cesarean section performed and the onset of symptom, mean±SD (months)</td>
<td>33.35±28.32</td>
<td>31.19±31.88</td>
<td>0.75</td>
</tr>
<tr>
<td>Previous cesarean section and the time of excision of scar endometriosis, mean ± SD (months)</td>
<td>58.65±36.87</td>
<td>67.19±33.87</td>
<td>0.31</td>
</tr>
<tr>
<td>CA125 levels, mean±SD (U/ml)</td>
<td>26.46±19.41</td>
<td>55.69±36.78</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Extent of lesions (n=110)*

<table>
<thead>
<tr>
<th></th>
<th>Non-mesh use case</th>
<th>Mesh use case</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Within the adipose layer with/without the skin layer, n (%)</td>
<td>46 (50.0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>- Within both the adipose and fascia layers with/without the skin layer, n (%)</td>
<td>28 (30.4%)</td>
<td>4 (22.2%)</td>
<td>0.67</td>
</tr>
<tr>
<td>- Included the adipose, fascia and rectus abdominis muscle layers</td>
<td>10 (10.9%)</td>
<td>6 (33.3%)</td>
<td>0.01</td>
</tr>
<tr>
<td>- Included the adipose, fascia, rectus abdominis muscle and peritoneum layers with/without the skin layer, n (%)</td>
<td>2 (2.2%)</td>
<td>6 (33.3%)</td>
<td>0.00</td>
</tr>
<tr>
<td>- Invaded both the fascia and rectus abdominis muscle layers, n (%)</td>
<td>2 (2.2%)</td>
<td>1 (5.6%)</td>
<td>0.65</td>
</tr>
<tr>
<td>- Invaded both the rectus abdominis muscle and peritoneum layers, n (%)</td>
<td>1 (1.1%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>- Invaded the fascia, rectus abdominis muscle and peritoneum layers, n (%)</td>
<td>3 (3.3%)</td>
<td>1 (5.6%)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

* There were a total of 110 abdominal endometriosis lesions among the 95 patients in this study, 92 (83.6%) in the non-mesh group and 18 (16.4%) in the mesh group.

torectomy (five in non-mesh used group and two in mesh group) were statistically different between mesh group and non-mesh group (Table 2). Cases for endometriosis lesions limited to the adipose layer had significant lower chance to use mesh (p < 0.001). However, adipose layer endometriosis lesions penetrating through the fascia layer and invaded into rectus abdominis muscle layer with/without peritoneum layer had a significantly higher chance to use mesh (p < 0.05) (Table 2).

The final pathological diagnosis for each lesion was AWE (Figure 1e). Most patients recovered well and symptoms that patients once complained of disappeared after surgery (Figure 1f). Only one patient presented with subcutaneous abscess after surgery and recovered well with drainage and antibiotic therapy.

Among the 95 patients, four patients were treated with gestrinone for three month before surgery and all reported a relief of severe pain and reduction of the mass; however, they all returned to the hospitals in six months due to the recurrence of pain and increasing mass after medication therapies. Two patients complained of one failed AWE surgery in other hospitals within two years and did not experience recurrence again after mass resection in the present hospital. Follow-up information covered three months to 5.5 years by telephone or clinic examination. Seventeen patients lost to follow up. Unfortunately, one patient with an AWE mass of 4 cm in diameter located in the adipose layer reported a recurrence mass for 1.0 cm in diameter by ultrasound with cyclical pain 18 months after the first surgery among the other 93 patients treated initially in the present affiliated hospitals. The patient received abdominal wall mass resection again and the lesion confirmed to be endometriosis mass by pathology. Three years later there was no other incidence since the second surgery performed in the present hospital without medication therapy.

**Discussion**

AWE may be a more common extrapelvic endometriosis than reflected in the literature in women of reproductive age [5]. In the present study, the average age of the patients with AWE was 33 years (range 26-48), in agreement with other studies [6, 7]. The incidence of AWE after cesarean section in the present study parallels the results in the published literature and was between 0.03% and 1% [8-10]. All patients in this study had a history of at least one prior cesarean section and presented one to three abdominal wall masses that developed in a cesarean section scar. One patient even presented an AWE lesion extending from the subcutaneous tissue to anterior wall of the uterus. The opinion that cesarean section is a leading risk factor for the developing of AWE is well supported and a surgically-induced ectopic endometrial implantation theory is proposed [11, 12].

It is well-known that gradually growing nodular abdominal mass in or adjacent to their cesarean incision scars is an important symptom in the diagnosis of AWE. Ozel et al. [11] reported that 72.2% masses were found at the left margin of the Pfannenstiel incision scars, while Teng et al. [13] described 13 out of the 19 foci (68.4%) that were located in right corner of the Pfannenstiel incision wounds. The present study parallels the findings that endometrial inoculation after cesarean section easily present at both corner sites [14], but no predominant corner can be identified given that many doctors were involved in the study. Though there is no logical explanation why abdominal scar endometriosis
is more commonly present at the corner of the incision wound, some reports assume that they may due to the following: the endometrial cavity is cleaned with sponge after placental removal, uterus incision is closed and the abdominal wound includes the same suture material, abdominal wound is not thoroughly irrigated with saline solution before closing the abdominal wall, and the choice of surgeon’s position may all contribute to the inoculum of endometriosis mass [13, 15].

Ding et al. [16] described that the fascia was frequently affected whereas the peritoneum was least commonly involved with regards to invasion of the abdominal layers by AWE (36.1% VS 15.0%). Liu et al. [17] demonstrated the incidence of AWE invading the peritoneum reached 66.7% when resecting and repairing large abdominal wall incisions. In contrast to the aforementioned literature, the most common site to find endometriosis was located in the adipose layer of the abdominal wall as in the present study. This may be due to the fact that each study has its own objects which resulted in bias options. These studies also applied different statistical methods during their research. In the present study, the occurrence one adipose layer endometriosis lesion penetrated through the fascia layer and invaded into rectus abdominis muscle layer with/without peritoneum layer, which had an increased possibility for mesh grafts to be used in the surgery.

Although exact reason remains unknown, there is usually a significant time delay between the onset of symptoms and abdominal wall mass resection surgery [18]. Similar to others studies [13, 16, 18], the time between symptom onset and mass surgery was 28 months in the present study. The typical symptoms of AWE are the periodic painful mass or swelling associated with menses. It is difficult to diagnose AWE before surgery as patients complained of non-cyclic painful abdominal wall without a painful palpable or hard mass, which may explain the time delay between the onset of symptoms and the abdominal wall mass resection surgery in a part of the cases. All patients had abdominal wall masses in the present study, including 89.5% of gradually growing painful masses (78.8% cyclical vs. 21.2% non-cyclical) which in agreement with others studies [19]. Patients with large abdominal wall masses may endure long term painful symptoms than with small ones as in the present study. Ozel L et al. [11] assumed that as the mass grows and reaches a larger size, the pain becomes more cyclical in nature, but it was not confirmed by the present group.

The pathogenesis of endometriosis related pain is complex and manifold. Barcena et al. [20] assumed that the actual cause of the endometriosis related pain may be due to a result of a neurogenic inflammatory reaction besides the peritoneal lesions and adhesions. Research was intensified on the occurrence of endometriosis-related nerve fibres when large quantities of nerve fibres were detected in endometriosis lesions and infiltrated by stroma cells [21]. Vercellini et al. [22] mentioned that there exists a neuropathic pain character as well as neurogenic inflammation processes besides the generally accepted inflammatory, nociceptive pain component, which was extremely particular when the initial cyclic pelvic pain becomes non-cyclic and/or chronic pain. The hypothesis merits further investigation for better understanding the pathogenesis of AWE related pain.

Owing to its practicality and low cost, ultrasonography is the most commonly used imaging examination performed to evaluate focal abdominal identified at physical examination. Images of AWE obtained by ultrasonography examination are non-specific in the literature. The typical ultrasound finding is a solid, non-homogeneous hypoechoic mass with spiculated margins infiltrating the surrounding tissue [10]. If ultrasound findings are inconclusive, MRI is suggested to determine the extent and nature of the focal lesions [23].

Fine-needle aspiration cytology is the initial approach for determining the nature of the mass and providing a diagnosis; however, its use remains challenging as it can cause abdominal cavity damage if an incision hernia had not been previously ruled out and has a potential diagnostic pitfall due to its rarity and occasional atypical cytological features [19, 24].

Although measurements of serum CA125 levels have been widely used in the gynecology field, the clinical significance of the serum CA125 elevation is still not fully understood in cases of endometriosis. The present study found that 7.4% of the patients with AWE had a history or subsequent diagnosis of pelvic endometriosis, which is within the range of the overall incidence of pelvic endometriosis in women of reproductive age [19]. This report also indicated that the serum CA125 level may increase in patients with large sizes of AWE. The present findings may offer some hints on the correlation between the size of the scar endometriosis and the value of the serum CA125 levels.

The malignant transformation of endometriosis in the abdominal wall is rare. No more than 30 cases have been reported in the literature [25]. The malignant type of endometriosis-associated neoplasm includes endometrioid carcinoma, sarcoma, and clear cell carcinoma. Malignancy transformation of endometriosis in the abdominal wall may occur in just a few months up to 18 years after surgery [10]. No malignant AWE were found among the present cases.

It is assumed in this report that medical therapy can temporary alleviation of symptoms, but cannot eliminate a mass, which is similar to that described by other authors [13, 14, 19]. A local wide excision of the scar endometriosis with at least one cm margin is recommended, although no studies have evaluated whether the surgical margin width affects the recurrence rate. The possible recurrence after surgery described in the literature reached 4.3% [19]. The present authors had less higher recurrence (1.1%) due to adequate surgical excision, even resection of the muscle and fascia, and peritoneal elements of the
abdominal wall in order to obtain a good outcome. The mean diameter of the mass was 3.25 cm in the present series with an average of 4.97 cm in the mesh group by ultrasound. Based on the largest diameter of the lesions, Rodrich et al. [26] have classified tissue loss into small size (< five cm), intermediate size (five to 15 cm) and large size (> 15 cm) defects because the size of the defect has a major impact on the surgical therapy. Eltayeb et al. [27] mentioned in their study that cases with large ventral hernias (> four cm) could not obtain primary closure. The present authors’ experience suggests that when the diameter of the abdominal wall mass determined by ultrasound examination is more than five cm larger, surgeons should be prepared for the possibility of abdominal wall defects and patients should be counseled that mesh repair may be necessary before surgical treatment.

Some suggestions for preventing AWE based on the implantation theory had been given in the literatures, such as using a wound edge protector to separate the edges of the incision, lifting the uterus outside of the pelvis before making the uterine incision, not using a sponge to clean the endometrial cavity, not using the same instruments and/or gloves which have touched endometrium, avoiding penetrating through endometrium layers when suture the uterine muscle, using separate needles for the abdominal and uterine closure, careful flushing and irrigating abdominal incision before closure, and extending the breastfeeding time to delay menstruation [12, 19, 20]. Above all, avoiding unnecessary cesarean section is recommended for preventing AWE.

Weakness of the present study included not using dimension as parameter to compare the difference between mesh and non-mesh use groups for short of information to calculate the exact size of the fascia defects during surgery. Other limitations included not comparing the relationships between the size and depth of the lesions and transverse and longitudinal incisions separately for having little mesh use cases in the present study. However, the present study provides some promising information for AWE diagnosis and therapy, but is limited by the fact that it is a single consultant retrospective non-randomised study. A case-control study should be used, the number of AWE cases needs to be increased, and more information should be collected in pre-, peri-, and postoperation in the future to authenticate the relationships between the size and depth of the AWE and the moment for mesh use.

Conclusion

AWE is a relatively common disease among women of reproductive age with a history of cesarean section and usually is underestimated by doctors and patients. The more common position for scar endometriosis may be in the adipose layer at the corner of the surgical scar. Periodic painful palpable scar mass with a cesarean section history strongly suggests the diagnosis of AWE. Large size of scar endometriosis may accompany with higher serum CA125 level at the same time. Wide local excision with adequate margins is recommended for the treatment of AWE regardless if they are the primary or recurrent lesions. Mesh therapy should be considered before surgery when the diameter of the abdominal wall mass detected by ultrasound is more than five cm larger and/or the lesions invades into rectus abdominis muscle with/without peritoneum tissues from both adipose and fascia layers.

References

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Application of fluorescence in situ hybridization (FISH) as a tool to aid cytogenetics in 1,409 fetal samples

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Summary
Aim: To evaluate the technical application of fluorescence in situ hybridization (FISH) as a support to classical cytogenetic in numerical chromosomal aneuploidies studies in samples of amniotic fluid, chorionic villus, and fetal loss. Materials and Methods: The authors performed cytogenetic analyses in 1,409 patients (678 amniocentesis, 512 chorionic villus samples, and 219 spontaneous abortions) during one year. FISH molecular study aided traditional cytogenetic in 90 cases. These cases were indicated based on the diagnostic hypothesis of each patient or when no cellular growth was obtained. The authors standardized the FISH in discoloured slides. Results: They had 85% positive FISH in amniotic fluid, 70% in chorionic villus, and 90% in abortion material using 13, 18, 21 X and Y centromeric probes. It showed 12% of altered FISH in amniotic fluid (100% trisomies), 10% in chorionic villus (50% trisomy and 50% X - monosomy), and 22% in abortion material (50% trisomy, 25% X-monosomy, and 25% triploidy). FISH and cytogenetic analysis confirmed the results. Conclusion: This technique revolutionized clinical and research applications of cytogenetics. In this particular paper, FISH was a valuable and reliable technique to promptly identify rapid detection of aneuploidies in interphase cells, metaphase spread and paraffin-embedded samples. It is hoped that, in the future, the economic viability of array CGH and FISH, with the decreasing cost of testing and their genomics advantages can be incorporated as routine and customized in the approach of prenatal diagnosis.

Key words: Cytogenetic; Fluorescence in situ hybridization; Prenatal diagnosis; Spontaneous abortions.

Introduction
Cytogenetic prenatal diagnosis is utilized to detect chromosomal abnormalities in the fetus and it has been considered a safe and reliable method, which has been recognized for more than 30 years, mainly for pregnant women at increased risk to chromosomal abnormality [1-5]. These chromosome abnormalities are responsible for more than dozens identifiable syndromes, being more common than the monogenic Mendelian disorder. It is estimated that they affect 0.7% of live births, 2% of pregnancies at an older age, and 50% of spontaneous abortions in the first trimester [1,5]. These data emphasize the importance of human karyotype study.

Chromosome banding techniques, particularly G-band, which have been available since the seventies, represented a considerable progress in the area of human cytogenetics. This method made it possible to identify all chromosome pairs and also the breaking point observed in most structural rearrangements [6-10].

Therefore, with the great advances in genetics, the first fetal medicine services appeared in the eighties in North American and European universities. In the nineties, in Brazil the genetics laboratories had began switching to this new area forming a multidisciplinary team made up of physicians (specialists in fetal medicine, ultrasonographers, obstetricians, pediatricians, neonatologists, geneticists, pathologists), biologists, psychologists, nurses, and others [11].

Consequently, fetal medicine commenced with a variety of different purposes and preventive, diagnostic or therapeutic actions aimed at protecting, assessing, and assisting fetal health. Patients referring to the Fetal Medicine services must comply with the following criteria: advanced maternal age (over 35 years old), family history of chromosome abnormalities, abnormalities detected by ultrasound, exposure to radiation and drugs, prenatal infections, and chromosome X-linked diseases [12].

Patients are referred to prenatal diagnosis for amniocentesis or biopsy of the chorionic villus, based on evidence of high fetal loss risk and the gestational week. Chorionic villus sampling is performed between the 9th and the 12th gestational weeks, early amniocentesis, between the 12th and the 14th gestational weeks, traditional amniocentesis between the 15th and 20th gestational weeks, and percutaneous umbilical cord blood sampling between the 24th and the 30th weeks [11].

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The FISH technique on human metaphase and interphase nuclei using DNA probes (sequences of nucleic acids labeled with fluorochromes that identify specific regions of complementary DNA in chromosomes fixed on slides) has become an indispensable tool for the study of physical human genomic cartography, as it has allowed a accurate regional chromosomal localization of single copy genes or DNA repeated sequences. Furthermore, the introduction of methods for marking non-isotopic DNA probes allowed this technique to be performed in any cytogenetic laboratory, since it does not require the use of radioactive material [13-15]. Thus, with FISH it was possible to establish an early diagnosis of the numerical chromosomal aneuploidies [13, 16-19] in those cases with indication of chromosome abnormalities and also in those inconclusive cytogenetic results due to inadequate cellular growth for the cytogenetic study.

In situ hybridization offers new and extraordinary possibilities for gene mapping. Currently, it is possible to map any gene or DNA sequence that has been cloned. An additional advantage of this technique is the fact that it can be used to detect alterations in the metaphase and prometaphase, as well as in the interphase nuclei, and in paraffin-embedded material [13].

The application of FISH as an aid to cytogenetics is the fundamental importance in order to obtain a fast diagnosis of the numerical chromosomal aberrations, in amniotic fluid, and chorionic villus [20-23], as well as in spontaneous abortions and fresh or paraffin-embedded samples [14, 20-24].

The purpose of this study was to evaluate the technical application of FISH as an aid to cytogenetics in the study of the numerical chromosomal aneuploidies in samples of amniotic fluid, chorionic villus, and fetal loss, mainly in patients with increased risk to abnormalities chromosomal.

Materials and Methods

The sample consisted for 1,409 patients from the “Hospital São Paulo” and “Centro Paulista de Medicina Fetal”, indicated for cytogenetic study, during one-year period. The cytogenetic analysis was performed in the laboratory of the “Centro Paulista de Medicina Fetal”. Ethic Committees in Research of UNIFESP/Hospital São Paulo by CEP number 0559/02.

From the total samples (1,409), 678 correspond to patients that were submitted to amniocentesis for cytogenetic study of the amniotic fluid; 512 correspond to patients submitted to biopsy of the chorionic villus and 219 correspond to spontaneously aborted products. Invasive prenatal testing was performed for the following indications: advanced maternal age, fetal abnormalities on ultrasound scanning, abnormal triploid test (alpha-fetoprotein, hCG, unconjugated estriol), previous fetal abnormality, and maternal anxiety (usually because family history of malformations or aneuploidies, only advanced maternal age).

The cytogenetic study was implemented in 100 cases by FISH molecular study, indicated on the basis of the diagnostic hypotheses of each patient, such as: family history of chromosome abnormalities, abnormalities detected by ultrasound, suggestive of chromosome mainly aberrations (like 21, 13 or 18 trisomy) and in those cases where no cellular growth was obtained or hydatidiform mole.

**FISH Protocol**

The FISH technique was developed based on the studies of Speleman et al. [17], Kuchinka et al. [25], Eiben et al. [26], Shulman et al. [23], and Jobanputra et al. [27, 28], and standardized with some modifications of the Cytocell kit.

**Amniotic fluid**: Amniocentesis was performed between the 14th and 24th gestational weeks. By using ultrasound guidance, an average of 18 to 20 ml samples of amniotic fluid were collected and sent to the laboratory for centrifugation the fluid in two 15 ml tubes at 1,500 rpm in an eight-tube centrifuge. The supernatant was partially rejected, leaving two ml in each tube, which were homogenized and precipitated. The material from one of the tubes was placed in a culture medium for karyotyping and the other was used for FISH. To the latter, a hypotonic solution of 0.8% sodium citrate was added and left for ten minutes in an oven at 37°C and then centrifuged. A five-ml methyl acetate (3:1) fixing solution was added to the precipitate and the material centrifuged for five minutes. This fixation was repeated twice. From the third fixation, three ml were reserved for dropping on previously iced slides and then placed on a water-bath at 60°C. The slides were left in an oven at 60°C for two hours for the following FISH pretreatment.

**Chorionic villus**: Chorionic villus biopsy was performed between the 11th and the 13th gestational weeks. The material was aspirated with ultrasound guidance and sent to the laboratory, where the chorionic villus was dissected with the aid of a stereomicroscope in order to start the direct preparation of the material. 0.1 ml of colcemid was added for 50 minutes to the material previously placed in the culture flask in an amniomax medium. After this period, the medium was removed and three ml hypotonic 1% sodium citrate was added, and the material placed in an oven at 37°C for five minutes.

A drop of fixing solution of methanol/acetic acid (3:1) was added, and the mixture left for five minutes at room temperature. The supernatant was removed, three ml of fixing solution were added, and the mixture left for ten minutes. The fixing of the material to the slides was then started using a plate heated at 45°C. After fixation, the slides were placed in an oven at 60°C for two hours moving on to item “a” below for the pretreatment of the slides.

Previously stained slides for cytogenetic analysis: The slides were discoloured by three increasing ethanol concentrations (70%, 85%, and 90%), and afterwards pretreatment was started.

“a” - pretreatment of the slides: The slides were incubated in 2xSSC (sodium citrate and sodium chloride solution) for 30 minutes at 37°C, dehydrated by increasing ethanol concentrations (70%, 85%, and 95%) for two minutes and left to dry at room temperature. After this stage, they were incubated with 70%/2xSSC formaldehyde from three to five minutes in a water bath at 65°C for DNA denaturation. Then they were passed through increasing chilled ethanol concentrations (70%, 85%,95%) for two minutes and were left to dry at room temperature. Hybridization: ten ml of the probe was placed on each slide using an Eppendorf tube, and were left in an oven at 37°C along with the sample slides and cover slips for two minutes; after the probe was applied onto the sample and covered with the cover slip.

The sides of the cover slip were sealed with rubber cement and left for five minutes in the dark at 72°C. After that the slides were placed in a humid chamber in an oven at 37°C from 16 to 18 hours. After hybridization, the rubber cement was removed with tweezers and the slides were incubated in 0.4xSSC pH 70,
Application of fluorescence in situ hybridization (FISH) as a tool to aid cytogenetics in 1,409 fetal samples

with Tween in a water-bath at 72°C for five minutes without shaking. The slides were incubated in 2xSSC pH 7.0 for five minutes with shaking (~120 rpm) and finally placed in a 1xPBD (phosphate buffered detergent) solution for two minutes at room temperature. Finally they were stained with 15 ml DAPI or propidium iodate, covered with cover slips and sealed with enamel. The slides were left in the dark for 30 minutes before being analyzed in a fluorescence microscope, equipped with individual filters for FITC, DAPI, and PI. From 100 to 150 nuclei were examined and the digitalized images were examined through the Q-FISH software.

Results

For the FISH analysis, 100 samples were selected, represented by amniotic fluid, chorionic villus, and spontaneous abortions. 81% of success was obtained, with 86% normal and 14% positive for aneuploidies (Table 1).

Using centromeric probes of chromosomes 13, 21, 18, X, and Y, trisomies were found in 70% of FISH analyses of the various types of materials, followed by X monosomy in 20% and triploidy in 10%. Trisomy 21 was the most frequent in amniotic fluid and in the chorionic villus, while trisomy 13 was more frequent in spontaneous abortions, beyond triploidy and X monosomy (Table 2).

Figure 1A represents the result of FISH in amniocytes with the chromosome 13/21 probe and Figure 1B shows the result using the centromeric probes of chromosomes 18, X, and Y, stained with DAPI. The indication for this analysis was the advanced maternal age (AMA = 40 years old) with the presence of the ultrasound marker (golf ball) and TN= 3.4 mm, indicating risk of Down’s syndrome. No cellular growth was obtained in the amniotic fluid and, consequently, the authors performed FISH for chromosomes 13/21, 18, X, and Y. The result was normal for all investigated chromosomes.

Figure 1C shows the results of FISH in chorionic villus of interphase nuclei with a single signal of the centromeric probe of chromosome X, stained with propidium iodate. X monosomy, one signal for X and none for Y (×100). D) Visualization of the FISH sign in chorionic villus in interphase nuclei using the centromeric probes of X and Y. Nucleus stained with propidium iodate. X monosomy, one sign for X and none for Y (×100). D) Visualization of the FISH sign in prestained slides with interphase nuclei of abortion material. Probe 13•, probe 21• stained with propidium iodate. 13 Trisomy (×100).

Table 1. — Samples analyzed by the FISH technique and the respective success percentages.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nº of patients</th>
<th>Nº FISH positive</th>
<th>Nº FISH negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid</td>
<td>50</td>
<td>44</td>
<td>6</td>
</tr>
<tr>
<td>Chorionic villus</td>
<td>30</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Abortion material</td>
<td>20</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>83</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 2. — The distribution of the aneuploidies detected by FISH with the chromosomes probes (13, 21, 18, X, and Y), normal FISH and maternal age.

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>Nº of patients</th>
<th>Trisomy 21</th>
<th>Trisomy 13</th>
<th>Trisomy 18</th>
<th>X monosomy</th>
<th>Triploidy</th>
<th>Normal FISH</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>52</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>21-25</td>
<td>236</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>26-30</td>
<td>327</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<td>21</td>
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<td>31-35</td>
<td>355</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>30</td>
<td>33</td>
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<tr>
<td>36-40</td>
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<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>16</td>
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<td>41-45</td>
<td>109</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>46-48</td>
<td>15</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1409</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>71</td>
<td>83</td>
</tr>
</tbody>
</table>
trisomy 13. This result was confirmed by the anatomic-pathological analysis.

In one of the samples of amniotic fluid that had inadequate cellular growth for karyotype analysis, the patient was with 25th gestational week and she presented ultrasound signs compatible with Patau syndrome (trisomy 13). In this case the FISH technique with probes for chromosomes 13 and 21 confirmed trisomy 13.

Discussion

In the last 30 years, the prenatal diagnosis with conventional cytogenetic analysis has been recognized as a safe and reliable method to determine chromosomal abnormality for couples with increased risk. However, in Brazil, lately FISH has played an important role in aiding cytogenetics in the identification of aneuploidies in prenatal diagnosis. The advent and development of new hybridization kits minimized many problems with the efficiency from FISH techniques in interphase nuclei [29-31].

Van Lijnschoten et al. [32], using centromeric probes of chromosomes 1, 16, 18, X, and Y in paraffin-embedded samples from abortions, concluded that FISH could be used in retrospective studies on this type of material, although the results with fresh material were better.

Jobanputra et al. [27, 28] reported 80% to 100% of success by applying FISH techniques in slides of amniotic fluid and chorionic villus, using centromeric probes of chromosomes 13, 18, 21, X, and Y. Of these results, 94% were normal, 6% altered and, of these, 97.3% were trisomy of chromosome 21. This study clearly shows the ability of FISH in detecting chromosome abnormalities in high risk pregnancies.

The present analysis showed that the results in samples of amniotic fluid, chorionic villus, and abortion material with respect to the success of the method, the results, and the importance of FISH to assist conventional cytogenetics in prenatal diagnosis were in accordance with Bryndorf et al., [33], Jobanputra et al. [28], Hsieh et al. [34], Moraes et al. [35], Tavokina et al. [36], Jovanovic et al. [37], and Braha et al. [38].

In amniotic fluid and chorionic villus the trisomy of chromosome 21 was the most frequent and in abortion material it was that of chromosome 13. These results were also confirmed by the literature [26, 28, 39], with the exception of the abortion material, since the centromeric probes of chromosomes 16, 22, and 15, were not included in the present analysis due to their the high cost.

For abortion material, also due to cost restrictions, Paradinas et al. [40] proposed FISH application only for the suspected cases of hydatiform moles. In the present study, the authors realize the importance of FISH in paraffin material abortion, especially in cases where there was not cell growth (15%) and the results from the pathology suggested the presence of hydatiform mole. To solve this paradigm the present authors will be studying these cases separately in the near future (Lewis et al., 2013) [41].

Today, with the enormous advances with cytogenomics, the technique CGH array can be offered to specific cases in which there are morphological changes in ultrasound and normal karyotype. This technique enables the detection of gains and losses of small regions of genetic material that are not visualized by conventional cytogenetics [42-44]. Cytogenetics analyses using banding techniques can identify chromosome deletions and duplications in the range of 5–10 Mb, the higher resolution provided by microarrays can detect changes as small as 50–100 kb [23].

The application of molecular biology techniques has revolutionized the areas of science and technology, mainly in the field of fetal medicine, as a tool for cytogenetic study in the prenatal diagnosis of approximately 12% of the cases. This application has allowed the indication of safe results, mainly concerning the detection of numerical chromosomal aneuploidies through FISH for those cases with indication of chromosome abnormalities or where no cellular growth was obtained, thus avoiding a new harvesting of material.

It is hoped that in the future, the economic viability of array CGH and FISH, with the decreasing cost of testing and its advantages can become effective and the genetic and genomic data can be incorporated to customize the approach of prenatal diagnosis.

Acknowledgements

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References


Application of fluorescence in situ hybridization (FISH) as a tool to aid cytogenetics in 1,409 fetal samples


[40] Lewis G.H., De Scipio C., Murphy K.M., Hale L., Beierl


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Evaluation of frequency of nausea and vomiting as well as depression level in pregnant women

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³ Eskisehir Osmangazi University, Medical Faculty, Public Health Department, Eskisehir; ⁴ Sakarya University, Medical Faculty, Department of Obstetrics and Gynecology, Sakarya; ⁵ Okan University, Faculty of Health Sciences, Istanbul (Turkey)

Summary

Objective: To determine the frequency of nausea and vomiting in pregnant (NVP) women, review associated factors, and evaluate the depression level. Materials and Methods: The study is a cross-sectional research conducted in pregnant women who applied to Sakarya Training and Research Hospital and Sakarya Maternity and Children Hospital between January 13, 2013 and March 23, 2013. The study group consisted of 606 pregnant women who were below 20 weeks gestation and agreed to take part in the study. The questionnaire form prepared in line with the study objective was completed by the pregnant women under supervision. The women who had a complaint of nausea and vomiting at least once a day during their pregnancy were deemed as “having a history of nausea and vomiting”. Rhodes index was used to evaluate the severity of nausea and vomiting. Depression level was evaluated with the Beck Depression Inventory. Chi-square test and Spearman’s Correlation Analysis were used to analyze the data. Statistical significance value was accepted as \( p < 0.05 \). Results: The age of pregnant women in the study group ranged from 17 to 39 years (mean age: 25.55 ± 4.95). The frequency of having nausea and vomiting in the pregnant women was determined to be 35.1% (n = 213). The frequency of having nausea and vomiting was determined to be higher in those with a nuclear family, working women, those with a poor family income, those who used any contraception method before the pregnancy, and those who had a history of nausea and vomiting in their previous pregnancy(ies) \( (p < 0.05 \text{ for each}) \). The pregnant women with a history of nausea and vomiting reported that their complaints increased the most with the smell of food as well as perfume/cigarette/body odor. In the women with a history of nausea and vomiting, frequency of depression was significantly higher \( (p < 0.05) \). A positive relationship was found between the severity of nausea and vomiting and depression level \( (p < 0.05) \). Conclusions: Nausea and vomiting were determined to be a major health problem in pregnancy. Depression frequency was higher in those with a history of nausea and history. The severity of nausea and vomiting increased with higher depression levels. More detailed studies are required to determine the causes of NVP as well as the risk factors.

Key words: Pregnancy; Nausea and vomiting; Rhodes index; Depression.

Introduction

Nausea and vomiting in pregnancy (NVP) are an important pregnancy complication adversely affecting the women’s quality of life. While the severity of this complication (also called morning sickness, emesis gravidarum or pregnancy sickness) varies, its frequency ranges from 50% to 70% in the first trimester [1-4]. The symptoms start typically five to six weeks later than the last menstruation, peak around 8-12 weeks, and reduce gradually over time [2-5]. The symptoms range from mild nausea to severe nausea and vomiting [6]. In a study of Munch and Schmitz on severity of nausea and vomiting in pregnancy, 50-60.8% of the pregnant women defined it as mild, 28.4-33% reported it as moderate, and 10.8-17% reported it as severe [7]. Frequency of vomiting in pregnancy was reported to vary by the countries and ethnic groups [6, 8-10].

Hyperemesis gravidarum (HG) is a complication characterized with malnutrition because of nausea and vomiting, loss of weight by 5%, acid base imbalance, electrolyte imbalance, and ketonuria [11, 12].

Although many studies were conducted on nausea in pregnancy, its reason has not been fully explained yet [10, 13-16]. Some of the researches studying the etiology of NVP emphasized psychological factors. However, both conditions were stated to have a role on the etiology [10, 13, 17].

While thyroid gland disorders, abnormal beta-HCG levels, liver diseases, autonomic dysfunction, and psychological disorders may cause nausea and vomiting in pregnancy, parity, mother’s age, planned nature of the pregnancy, and lack of social support were also suggested to cause these complications [3, 18, 19]. Stress, insufficient information about the pregnancy and delivery, problems in family relationships, ambivalent feelings towards pregnancy, low sense of self-worth, lack of family and friend support, lack of acceptance of the wanted child, general sense of unhappiness, and concerns about fetal...
nutrition may also cause nausea and vomiting in pregnancy [20].

The pregnant women with dehydration and malnutrition due to severe nausea and vomiting whose clinical picture does not improve despite of the treatment efforts also need psychological support [4, 21]. The individuals with nausea and vomiting in pregnancy need professional support. As the etiological factors of nausea and vomiting in pregnancy are not clear, the symptomatic approaches are used. However, the symptomatic approaches fail to fully resolve the problem. Therefore, the etiological factors should be known so as to help pregnant women to deal with nausea and vomiting in pregnancy [19]. The healthcare professionals providing antenatal care should provide the pregnant women with information on pregnancy, delivery, pregnancy complications, and psychological changes during pregnancy. Thus, it can be ensured that the pregnant women would realize their problems and feel better [22]. Particularly, the healthcare professionals should be able to use available resources of social aids for the care of mothers and infants and ensure that these resources are increased when needed. The midwives and nurses can therefore benefit from the resources of social aids for the pregnant women and assist them to cope with their problems [19].

This study was conducted to determine the frequency of nausea and vomiting, review associated factors, and evaluate the depression level in pregnant women.

Materials and Methods

The study is a cross-sectional research conducted on the pregnant women who applied to Sakarya Training and Research Hospital and Sakarya Maternity and Children Hospital between January 13, 2013 and March 23, 2013.

The number of pregnant women presenting to the pregnancy follow-up polyclinic in a month is 2,500, with a daily average of 120 to 150 pregnant women presenting to the pregnancy follow-up polyclinic at the 11-bed maternity ward of Sakarya Training and Research Hospital and Sakarya Maternity and Children Hospital.

The questionnaire form prepared in line with the study objective included questions on some socio-demographic characteristics of the pregnant women with and without the history of nausea and vomiting, review associated factors, and evaluate the depression level in pregnant women.

### Table 1. Some socio-demographic characteristics of the pregnant women with and without the history of nausea and vomiting

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>History of nausea and vomiting</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No n (%)*</td>
<td>Yes n (%)*</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 24</td>
<td>95 (64.2)</td>
<td>53 (35.8)</td>
</tr>
<tr>
<td>25-29</td>
<td>128 (64.39)</td>
<td>71 (35.7)</td>
</tr>
<tr>
<td>30-34118 (66.7)</td>
<td>59 (33.3)</td>
<td>177 (29.2)</td>
</tr>
<tr>
<td>≥ 35</td>
<td>52 (63.4)</td>
<td>30 (36.6)</td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school and lower</td>
<td>133 (64.6)</td>
<td>73 (35.4)</td>
</tr>
<tr>
<td>Secondary school</td>
<td>102 (72.99)</td>
<td>38 (27.1)</td>
</tr>
<tr>
<td>High school</td>
<td>91 (59.5)</td>
<td>62 (40.5)</td>
</tr>
<tr>
<td>University</td>
<td>67 (62.6)</td>
<td>40 (37.4)</td>
</tr>
<tr>
<td>Family type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear</td>
<td>300 (62.5)</td>
<td>180 (37.5)</td>
</tr>
<tr>
<td>Extended</td>
<td>93 (73.8)</td>
<td>33 (26.2)</td>
</tr>
<tr>
<td>Employment status</td>
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<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>310 (68.4)</td>
<td>143 (31.6)</td>
</tr>
<tr>
<td>Employed</td>
<td>83 (54.2)</td>
<td>70 (45.8)</td>
</tr>
<tr>
<td>Family income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>25 (47.2)</td>
<td>28 (52.8)</td>
</tr>
<tr>
<td>Moderate</td>
<td>288 (68.7)</td>
<td>131 (31.3)</td>
</tr>
<tr>
<td>Good</td>
<td>80 (59.7)</td>
<td>54 (40.3)</td>
</tr>
<tr>
<td>Social security status</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>41 (71.9)</td>
<td>16 (28.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>352 (64.1)</td>
<td>197 (35.9)</td>
</tr>
<tr>
<td>Personality type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>239 (65.3)</td>
<td>127 (34.7)</td>
</tr>
<tr>
<td>B</td>
<td>154 (64.2)</td>
<td>86 (35.8)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>329 (64.1)</td>
<td>184 (35.9)</td>
</tr>
<tr>
<td>Yes</td>
<td>55 (70.5)</td>
<td>23 (29.5)</td>
</tr>
<tr>
<td>History of a physician-diagnosed chronic disease</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>352 (66.0)</td>
<td>181 (34.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>41 (56.2)</td>
<td>32 (43.8)</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>311 (65.9)</td>
<td>161 (34.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>82 (61.2)</td>
<td>52 (38.8)</td>
</tr>
<tr>
<td>Total</td>
<td>393 (64.9)</td>
<td>213 (35.1)</td>
</tr>
</tbody>
</table>

* Percentages were calculated based on the line total; ** Percentages were calculated based on the column total.
take part in the study constituted the study group. The pregnant women were interviewed in the waiting room of the hospitals. The pregnant women were informed about the subject and objective of the study, and their verbal consents were taken. Previously prepared questionnaire forms were completed by the pregnant women who agreed to take part in the study under supervision. This procedure lasted for approximately 15-20 minutes.

The women who had a “complaint of nausea and vomiting at least once a day during their pregnancy” were deemed as having a history of nausea and vomiting in the present study. The symptomatology of NVP ranges from mild to severe according to Rhodes et al. [23]. Such scoring system for nausea and vomiting was based mainly on a sample of patients receiving cancer chemotherapy. The Turkish version of Rhodes’ Score developed for research purposes has highlighted the substantial psychosocial morbidity of nausea and vomiting [24]. However, this system has been validated only for symptoms that occurred in the past 12 hours [25]. Eight questions were asked of patients and Rhode’s score can range from 8 (no symptoms) to 40 (maximal symptoms). Pregnant women who had scores lower than 9 were group 1, mild NVP ranging from 9 to 18, group 2, a moderate NVP from 19 to 32, group 3, and severe NVP score above 32, group 4. Depression level in this study was evaluated with the Beck Depression Inventory. The BDI was developed by Beck et al. in 1961 and later modified by Hisli in 1999 to suit the Turkish culture and norms [26, 27]. It is a 21-item self-report inventory on a four-point Likert scale. The inventory scores ranged between 0 and 63 and those with a score of 17 and above were regarded to have “suspected depression”.

The women who had any income-generating job (e.g. worker, civil servant, farmer, self-employed etc.) were defined as “employed”. The self-perceived family income level was assessed as high, medium, and poor by the patients. Those who defined themselves as uptight, enthusiastic, hasty, and impatient among the pregnant women were classified in “Type A personality” and those who defined themselves as quiet, calm, patient, and organized were classified in “Type B personality”[28]. Pregnant women who smoked at least one cigarette per day were defined as smokers, whereas nonsmokers were defined as women who had never smoked or who had not smoked in the past six months [29]. Menstruation with equal intervals (from 21 to 35 days) in the period before pregnancy was considered regular menstruation.

Obtained data were assessed with SPSS (version 20.0) Statistical Package Program, Chi-squared test, and Spearman’s Correlation Analysis were used for the analyses. Statistical significance value was accepted as $p < 0.05$.

### Table 2. — Some obstetric and gynecological characteristics of the pregnant women with and without the history of nausea and vomiting.

<table>
<thead>
<tr>
<th>Obstetric/gynecological characteristics</th>
<th>History of nausea and vomiting</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No n (%)*</td>
<td>Yes n (%)*</td>
</tr>
<tr>
<td>Number of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>164 (67.5)</td>
<td>79 (32.5)</td>
</tr>
<tr>
<td>1</td>
<td>134 (61.2)</td>
<td>85 (38.8)</td>
</tr>
<tr>
<td>2</td>
<td>65 (60.7)</td>
<td>42 (39.3)</td>
</tr>
<tr>
<td>3 and more</td>
<td>30 (81.1)</td>
<td>7 (18.9)</td>
</tr>
<tr>
<td>Number of pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>145 (65.6)</td>
<td>76 (34.4)</td>
</tr>
<tr>
<td>2</td>
<td>134 (63.2)</td>
<td>78 (36.8)</td>
</tr>
<tr>
<td>3 and more</td>
<td>114 (65.9)</td>
<td>59 (34.1)</td>
</tr>
<tr>
<td>Current pregnancy week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 7</td>
<td>78 (67.2)</td>
<td>38 (32.8)</td>
</tr>
<tr>
<td>8-15</td>
<td>190 (64.2)</td>
<td>106 (35.8)</td>
</tr>
<tr>
<td>≥ 16</td>
<td>125 (64.4)</td>
<td>69 (35.6)</td>
</tr>
<tr>
<td>Wanted pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>37 (66.1)</td>
<td>19 (33.9)</td>
</tr>
<tr>
<td>Yes</td>
<td>356 (64.7)</td>
<td>194 (35.3)</td>
</tr>
<tr>
<td>Fertility treatment- induced pregnancy</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>351 (64.4)</td>
<td>194 (35.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>42 (68.9)</td>
<td>19 (31.1)</td>
</tr>
<tr>
<td>Use of drugs in current pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>270 (63.8)</td>
<td>153 (36.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>123 (67.2)</td>
<td>60 (32.8)</td>
</tr>
<tr>
<td>Use of contraceptive method before pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coitus interruptus</td>
<td>76 (59.8)</td>
<td>51 (40.2)</td>
</tr>
<tr>
<td>Condom</td>
<td>80 (57.1)</td>
<td>60 (42.9)</td>
</tr>
<tr>
<td>IUD</td>
<td>34 (69.4)</td>
<td>15 (30.6)</td>
</tr>
<tr>
<td>Oral contraceptive</td>
<td>45 (60.0)</td>
<td>30 (40.0)</td>
</tr>
<tr>
<td>History of nausea-vomiting in previous pregnancies (n = 394)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>128 (74.0)</td>
<td>45 (26.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>126 (57.0)</td>
<td>95 (43.0)</td>
</tr>
<tr>
<td>History of gynecological surgery</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>349 (65.4)</td>
<td>185 (34.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>44 (61.1)</td>
<td>28 (38.9)</td>
</tr>
<tr>
<td>Menstrual regularity before pregnancy</td>
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<td></td>
</tr>
<tr>
<td>Irregular</td>
<td>77 (64.2)</td>
<td>43 (35.8)</td>
</tr>
<tr>
<td>Regular</td>
<td>316 (65.0)</td>
<td>170 (35.0)</td>
</tr>
<tr>
<td>Total</td>
<td>393 (64.9)</td>
<td>213 (35.1)</td>
</tr>
</tbody>
</table>

*: Percentages were calculated based on the line total; **: Percentages were calculated based on the column total.
Table 3. — The distribution of the drugs used by the pregnant women during pregnancy.

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron supplement</td>
<td>314</td>
<td>47.4</td>
</tr>
<tr>
<td>Vitamin</td>
<td>299</td>
<td>45.1</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>7</td>
<td>1.1</td>
</tr>
<tr>
<td>Analgesics</td>
<td>25</td>
<td>3.8</td>
</tr>
<tr>
<td>Insulin</td>
<td>13</td>
<td>1.9</td>
</tr>
<tr>
<td>Antiemetic</td>
<td>5</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>663</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4. — The distribution of the factors which increase nausea and vomiting in pregnancy.

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food smells</td>
<td>154</td>
<td>38.0</td>
</tr>
<tr>
<td>Weakness</td>
<td>70</td>
<td>17.3</td>
</tr>
<tr>
<td>Perfume/cigarette/body odor</td>
<td>137</td>
<td>33.8</td>
</tr>
<tr>
<td>Special foods</td>
<td>44</td>
<td>10.9</td>
</tr>
<tr>
<td>Total</td>
<td>405</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 5. — Some characteristics of the pregnant women with and without suspected depression.

<table>
<thead>
<tr>
<th>Nausea-vomiting characteristics</th>
<th>Suspected depression</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No n (%)*</td>
<td>Yes n (%)*</td>
</tr>
<tr>
<td>History of nausea-vomiting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>352 (89.6)</td>
<td>41 (10.4)</td>
</tr>
<tr>
<td>Yes</td>
<td>167 (78.4)</td>
<td>46 (21.6)</td>
</tr>
<tr>
<td>Total</td>
<td>519 (85.6)</td>
<td>87 (14.4)</td>
</tr>
<tr>
<td>Pregnancy month in which nausea and vomiting occur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 1</td>
<td>113 (79.6)</td>
<td>29 (20.4)</td>
</tr>
<tr>
<td>Month 2</td>
<td>33 (76.7)</td>
<td>10 (23.3)</td>
</tr>
<tr>
<td>Month 3 and later</td>
<td>21 (75.0)</td>
<td>7 (25.0)</td>
</tr>
<tr>
<td>Total</td>
<td>167 (78.4)</td>
<td>46 (21.6)</td>
</tr>
<tr>
<td>Time of day when nausea and vomiting peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>85 (76.6)</td>
<td>26 (23.4)</td>
</tr>
<tr>
<td>Evening</td>
<td>21 (75.0)</td>
<td>7 (25.0)</td>
</tr>
<tr>
<td>All day</td>
<td>61 (82.4)</td>
<td>13 (17.6)</td>
</tr>
<tr>
<td>Total</td>
<td>167 (78.4)</td>
<td>46 (21.6)</td>
</tr>
</tbody>
</table>

* Percentages were calculated based on the line total; ** Percentages were calculated based on the column total.

Results

The study group consisted of 606 pregnant women aged from 17 to 39 years (mean age: 25.55 ± 4.95). Of the pregnant women, 148 (24.4%) were aged 24 and below, 199 (32.8%) were aged 25-29, 177 (29.2%) were aged 30-34, and 82 (13.5%) were aged 35 and above. In the present study, the frequency of having nausea and vomiting in the pregnant women was determined to be 35.1% (n = 213).

Some socio-demographic characteristics of the pregnant women in the study group with and without the history of nausea and vomiting are given in Table 1.

243 (40.1%) women in the study group have not given birth before and it was the first pregnancy in 221 (36.5%) women. The number of women who have not used a contraceptive method before pregnancy was 215 (35.5%). The distribution of the pregnant women with and without a history of nausea and vomiting by some obstetric and gynecological characteristics is given in Table 2.

In the study group, the most commonly used drugs during pregnancy were iron supplements (47.4%) and vitamins (45.1%). The distribution of the drugs used by the pregnant women during pregnancy is given in Table 3.

Smell of food (38.0%) and perfume/cigarette/body odor (33.8%) were among the most frequently reported factors which increased NVP. The distribution of the factors which increase this complaint in study population with a history of nausea and vomiting is given in Table 4.

Suspected depression was diagnosed in 87 pregnant women (14.4%) in this study. The presence of nausea and vomiting, pregnancy month in which nausea and vomiting occur, and the distribution of nausea and vomiting by the time of day when nausea and vomiting peak in the study population with and without depression are given in Table 5.

The scores obtained from Beck Depression Inventory by the pregnant women in this study ranged from 0 to 43, with a mean score of 8.60 ± 6.43. The scores obtained from Rhodes Index of Nausea and Vomiting ranged from 8 to 31 (mean score: 12.01 ± 5.64). A positive relationship was found between the severity of nausea and vomiting and depression level in the present study (\( r = 0.270; p = 0.000 \)). The distribution of the scores obtained from the index of nausea and vomiting and depression scale by the pregnant women is given in Figure 1.

Discussion

35.1% of the pregnant women in the present study reported nausea and vomiting complication. Some studies noted that the frequency of NVP ranged from 50% to 70% [30, 31]. Gadsby et al. stated that 63.2% of the pregnant
women had nausea and vomiting in their previous pregnancies [32]. In the study of Koken et al. conducted in Turkey, the frequency of nausea and vomiting was 72.9% [3]. Particularly, the changes in the sense of smell and taste during pregnancy cause the pregnant woman to develop an aversion to some foods and smells. This condition causes an increase in nausea and vomiting complication in the pregnant women and insufficient consumption of nutrients required for fetus [33].

The frequency of nausea and vomiting in pregnant women who are actively engaged with an income-generating job was determined to be higher in the present study \((p < 0.05)\). Koken et al. reported that nausea and vomiting increase with fatigue and the feeling of nausea and vomiting in pregnant women subsides when they rest. Other studies stated that the nausea and vomiting complication increased mostly in situations like intensive working environment and lack of sleep in pregnant women [3, 10]. It can be argued that working makes a person tired and thus increases nausea and vomiting.

Vomiting is one of the most common physical reactions to stress. Iatrakis et al. reported that somatic responses including vomiting were more common in pregnant women under stress [34]. As poor socio-economic status is a stress factor, it is expected to increase nausea and vomiting even more in pregnant women. Accordingly, the frequency of nausea and vomiting was observed to be higher in pregnant women with poor family income \((p < 0.05)\).

In the study of Koken et al. it was reported that there is no relationship between nausea and vomiting and number of pregnancies, deliveries, and abortions [3]. In the present study, no difference was found between the number of deliveries and pregnancies and frequency of nausea and vomiting in the study population \((p > 0.05)\) for each.

No difference was determined between the frequency of nausea and vomiting and planned or unplanned nature of pregnancy in the study population \((p > 0.05)\). Kuo et al. reported that the women with severe nausea and vomiting accept the pregnancy less than the women with mild to moderate nausea and vomiting [35].

In this study, the frequency of nausea and vomiting was found to be higher in women with a history of nausea and vomiting in their previous pregnancy/pregnancies \((p < 0.05)\). Timur et al. reported that the risk of having nausea and vomiting is higher in women with a history of nausea and vomiting in their previous pregnancy/pregnancies. Similarly, many studies claimed that a history of nausea and vomiting in previous pregnancy is a risk factor for having nausea and vomiting in current pregnancy [4, 31, 36].

Smells are one of the most important triggers of nausea and vomiting of pregnancy. Food (particularly meat), coffee, perfume, cigarette, and volatile substances (petroleum products) are the primary smells triggering nausea and vomiting in pregnant women. Hyperactive sense of smell triggered by estrogen level in early pregnancy may contribute to this condition [31, 37]. Consistently, the pregnant women in the present study reported that their complaints increased the most with the smell of food as well as perfume/cigarette/body odor. These results are consistent with the present study.

The frequency of manifesting depressive symptoms in pregnancy may vary by the pregnancy trimester. In the literature, anxiety and depression were reported to occur more in the first and third trimesters compared to the second trimester of the pregnancy [9, 38, 39]. The frequency of depression was determined to be significantly higher in women with a history of nausea and vomiting in the present study \((p < 0.05)\). Occurrence of anxiety and depression in the first trimester may be associated with more frequent nausea and vomiting in this period.

A positive relationship was found between the severity of nausea and vomiting and depression level \((p < 0.05)\). Ozen et al. stated that anxiety level was higher in the pregnant women with HG [30]. In another study, the frequency of anxiety and depression in the pregnant women with HG was reported to be higher compared to the pregnant women without this condition [40]. Kim et al. suggested that the quality of life significantly deteriorated in women with HG and such women should be observed in psychiatric terms [41].

Conclusions

Nausea and vomiting were determined to be a major health problem in pregnancy. The pregnant women reported that smell of food and perfume/cigarette/body odor increase...
nausea and vomiting. The frequency of nausea and vomiting was found to be higher in the women who used any contraceptive method before pregnancy. Depression frequency was higher in those with a history of nausea and history. The severity of nausea and vomiting increases with higher depression levels. It may be advantageous to perform depression screens in pregnant women and to refer suspected cases to advanced centers for definitive diagnosis and treatment. More detailed studies are required to determine the causes of nausea and vomiting in pregnancy as well as the risk factors.

Limitations

Cross-sectional nature of the study is one of the limitations of this study. Other limitations may include the facts that it was conducted in a single city and hospital and that the scales used for diagnosis of depression fail to provide a definitive diagnosis.

Acknowledgments

The authors wish to thank all the pregnant women and hospital staff who joined this study.

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Evaluation of frequency of nausea and vomiting as well as depression level in pregnant women

Comparison of different severities of nausea and vomiting during pregnancy relative to stress, social support, and maternal adaptation. J. Midwifery Womens Health, 2007, 52, 1.


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Predictive value of transvaginal ultrasound score for detection of endometrial malignancy

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Summary
Purpose: The aim of this study was to assess the accuracy of transvaginal ultrasound in detecting endometrial malignancy in perimenopausal women. Material and Methods: The cross-sectional study included 100 perimenopausal women who had changes on the endometrium discovered through a regular ultrasound check-up and were referred to Clinic of Gynecology and Obstetrics “Narodni Front” in Belgrade during the period from September 1, 2012 to September 1, 2013. Transvaginal ultrasound was performed on each participant in the study. Parameters of the ultrasound examination composed a score system. Result: The results of regression analysis showed that this transvaginal ultrasound score have independent prognostic value for detection of endometrial malignancy. Score system showed that the value 8 had the best validity for the detection of endometrial malignity, with the sensitivity of 0.857 and specificity of 0.785. Conclusion: The collected transvaginal ultrasound sample had high predictive value for the discovery of malign changes on endometrium.

Key words: Ultrasound; Perimenopause; Endometrial carcinoma; Metrorrhagia; Endometrial hyperplasia; Endometrial polyps; Endometrial thickness.

Introduction
Ultrasoundography is a method that allows visualization of endometrium and recognition of its pathology, and is a painless and non-invasive method that requires minimal time for a check-up and is acceptable by the majority of women [1, 2]. A reduced production of estrogen in perimenopause causes endometrium atrophy, which gives a sonographic picture of a narrow and intermittent structure. The major ultrasonicographic finding in females with pathological changes of endometrium is thickening of endometrium [2]. The results from numerous studies have confirmed that sensitivity for detection of pathological changes of endometrium according to the sonographic thickness is very low [3]. There are no reliable sonographic criteria to distinguish findings between benign and malign changes on the endometrium. The most frequent benign conditions are: endometrial polyp or endometrial hyperplasia. For both of these changes is a thickened endometrium of a homogenous appearance, unlike thickened endometrium of an inhomogeneous appearance that is found in atypical hyperplasia and endometrial carcinoma [4]. Among females between 45-55 years of age, the most frequent signs are disorders of menstrual cycle as an absence of menstruation or irregular bleeding from the uterus [5]. In this group of patients, ultrasound most frequently shows thickness of endometrium, endometrial polyp or there is a suspicion of a malign process in the endometrium [6]. An early diagnosis is an imperative for the prognosis of the patients suffering from endometrium carcinoma. To the best of the present authors’ knowledge, there is no sufficient valid screening test for detection of endometrial malignancy. The aim of this study was to establish transvaginal ultrasound score as well as to investigate its predictive value in detection of endometrial malignancy.

Materials and Methods
The survey was designed as a type of cross-sectional study. It was performed from September 1, 2012 to September 1, 2013, in the Clinic of Gynecology and Obstetrics “Narodni Front” in Belgrade, a referral centre for gynecology in Serbia. The study included 100 perimenopausal women, that had changes on the endometrium, discovered through a regular ultrasound check-up or the patients were directed to the clinic due to irregular bleeding from the uterus. The exclusion criteria were: previously confirmed malignant disease, myoma of the uterus, any adnexal pathology, and hematological disorder. All the patients had regular colposcopic and cytological finding in the last six months. Transvaginal ultrasound was performed on each participant in the study. Check-ups were done using ultrasonic apparatus with a five-MHz transvaginal probe. In each patient the uterine cavity was checked in the sagittal and the transversal planes.

The ultrasound examination was described by a selected group of parameters that composed a score according to which state the endometrium was evaluated. This score included the following...
parameters: thickness of endometrium (up to five mm = 0, from five to eight mm = 1, > eight mm = 2), echogenicity of the endometrium compared to the myometrium: normal echogenicity = 0, hyperechogenous = 1, hypechoegenous = 2, the border of the endometrium towards the myometrium - subendometrial hypechoegenous zone (whole = 0, intermittent = 1), homogeneity of the texture of the endometrium (homogenous = 1, inhomogeneous = 2), presence of the coloured signals in the endometrium (present = 2, absent = 1), index of resistance in newly-formed blood vessels of the endometrium (> 0.4 = 1, < 0.4 = 2), volume of the endometrium by an ultrasound check-up (< 13 ml = 1, > 13 ml = 2). All females in the present study had fractional explorative curetage and the obtained material was examined histopathologically. Before the intervention, in order to exclude the presence of malign changes on cervix, a cytological and colposcopic check-up was performed. Anaesthesia was mainly intravenous.

The primary analysis involved descriptive statistics summary for estimating demographic and clinical characteristics of the study participants. The differences between transvaginal ultrasound score among perimenopausal females with and without endometrial malignancy were assessed by using t-test. Independent predictors of endometrial malignancy were identified using a series of logistic regression models based on heterogeneous risk factors with potential confounding effects. All potential covariates were first analyzed in a univariate unadjusted regression model with occurrence of endometrial malignancy as dependent variable. Subsequently, a multivariate logistic regression analysis was performed to test whether possible predictors remained significant. This adjusted analysis included all covariates that appeared to be associated (p < 0.05) with the endpoint in the first analysis.

Receiver operating characteristic (ROC) curve analysis was used to determine a cut-off value of transvaginal ultrasound score for an identification of endometrial malignancy.

Results

Average age of participants was 50.6 ± 3.42 years. The majority of the participants (66%) were pluripara. Average number of deliveries was 1.74 ± 0.75. Twenty women had history of spontaneous abortion, while 27% had planned abortion. Eighteen females in this sample had a sterility problem. The average age of menarche was 12.11 ± 1.60 years. Regular menstrual cycle was present for 31% patients. Twenty-eight females had hypertension, 13% had dysfunction of thyroid gland, while 8% had diabetes. Forty-three (43%) women included in this investigation were smokers. One-third of patients had an obesity problem. The distribution of initial diagnosis before transvaginal ultrasound was as follows: endometrial hyperplasia (43%), endometrial polyp (37%), and with a diagnosis of suspicion of carcinoma (7%). Problems with uterine bleeding during the examination were present in 52% of participants. Histopathological analysis showed that 21% patients had malign and 79% benign pH finding. According to the data illustrated in Figure 1, the values of transvaginal ultrasound score in this sample of perimenopausal females ranged from 4 to 13. In the group of women with benign histopathological findings, the average value of this score was 6.96 ± 1.85 (range 4-11), while in the group of women with malignant histopathological findings, the average value of this score was 10.38 ± 1.86 (range 7-13). The differences between transvaginal ultrasound score among these two subcohorts was statistically highly significant (t = - 7.522; p < 0.001). The “gray zone” of overlapping values in these two groups ranged between 7 and 11. Table 1 labeled “sensitivity and specificity” tabulates those values for each possible cut-off between benign and malignant histopathological findings. The best validity of transvaginal ultrasound score for detection of endometrial malignancy was observed with the value of 8, specifying a sensitivity of 0.857 and specificity of 0.785.

The predictors of endometrial malignancy that were identified using logistic regression models are illustrated in Table 2. The unadjusted models have revealed that prognostic value for occurrence of endometrial malignancy had following variables: spontaneous abortion, hypertension, obesity, and transvaginal ultrasound score. Furthermore, after testing for variable interaction and controlling the ef-

Table 1. — Sensitivity and specificity values of transvaginal ultrasound score for each possible cut-off between benign and malignant histopathological findings.

<table>
<thead>
<tr>
<th>Positive if greater than or equal to (a)</th>
<th>Sensitivity</th>
<th>1 - Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.00</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>4.50</td>
<td>1.000</td>
<td>0.911</td>
</tr>
<tr>
<td>5.50</td>
<td>1.000</td>
<td>0.772</td>
</tr>
<tr>
<td>6.50</td>
<td>1.000</td>
<td>0.544</td>
</tr>
<tr>
<td>7.50</td>
<td>0.905</td>
<td>0.380</td>
</tr>
<tr>
<td>8.00</td>
<td>0.857</td>
<td>0.215</td>
</tr>
<tr>
<td>9.50</td>
<td>0.667</td>
<td>0.101</td>
</tr>
<tr>
<td>10.50</td>
<td>0.476</td>
<td>0.038</td>
</tr>
<tr>
<td>11.50</td>
<td>0.333</td>
<td>0.000</td>
</tr>
<tr>
<td>12.50</td>
<td>0.143</td>
<td>0.000</td>
</tr>
<tr>
<td>14.00</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 1. — Values of transvaginal ultrasound score according to the histopathological findings.
Predictive value of transvaginal ultrasound score for detection of endometrial malignancy

Effect of potential confounders, the multivariate adjusted model demonstrated that independent prognostic value for detection of endometrial malignancy remained significant only for spontaneous abortion and transvaginal ultrasound score. Namely, this analysis showed that women with spontaneous abortion in medical history had six-fold greater chance to develop endometrial cancer compared to females without this spontaneous abortion in medical history (OR = 6.17, \( p = 0.033 \)). Additionally, this predictive model also demonstrated that with each one-unit increase in transvaginal ultrasound score, the risk for detection of endometrial malignancy scale increased by 2.52-fold (OR = 2.52, \( p < 0.001 \)). The area under a ROC curve quantified the overall ability of the test to discriminate between those individuals with the disease and those without the disease (Figure 2). A truly useless test (one no better at identifying true positives than flipping a coin) had an area of 0.5. A perfect test (one that has zero false positives and zero false negatives) has an area of 1.0. In this investigation the area under the curve was 0.896, indicating an excellent effectiveness of transvaginal ultrasound score for the identification of endometrial malignancy.

**Discussion**

In the present sample of perimenopausal women, 31% had regular cycles in the last six months, which was consistent with the facts regarding irregularities and abnormalities of the perimenopause cycle.

Twenty-eight females suffered from hypertension, which is consistent with the result obtained by Litta et al. [7]. Thirteen of participants suffered from a thyroid dysfunction and 8% of all participants had diabetes [8]. In the present study risk factors, cigarette smoking, and obesity confirmed that these are risk factors for endometrial cancer [9-11]. In 35 patients, endometrial polyps were confirmed, therefore the interventions were justified. Due to the possibility of a malignancy within a polyp, numerous experts believe that such a change ought to be removed. [12]. The majority (52%) of women in the present investigation had endometrial bleeding during examination which is in agreement with previous findings that during perimenopause, the risk of creation of liggities on endometrium increased with abnormal bleeding [13].

**Table 2. — Logistic regression models of predictors of endometrial malignancy in premenopausal women.**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Unadjusted models</th>
<th>Adjusted model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.07</td>
<td>0.92 – 1.24</td>
</tr>
<tr>
<td>Menarche (years)</td>
<td>1.04</td>
<td>0.77 – 1.40</td>
</tr>
<tr>
<td>Parity</td>
<td>0.48</td>
<td>0.18 – 1.28</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>8.56</td>
<td>2.84 – 25.76</td>
</tr>
<tr>
<td>Intentional abortion</td>
<td>0.57</td>
<td>0.17 – 1.89</td>
</tr>
<tr>
<td>Treated sterility</td>
<td>1.30</td>
<td>0.49 – 3.43</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5.25</td>
<td>1.89 – 14.61</td>
</tr>
<tr>
<td>Thyroid disorders</td>
<td>1.83</td>
<td>0.50 – 6.65</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.01</td>
<td>0.24 – 2.35</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.93</td>
<td>1.44 – 10.71</td>
</tr>
<tr>
<td>Endometrial hyperplasia in obs.</td>
<td>0.45</td>
<td>0.16 – 1.29</td>
</tr>
<tr>
<td>Endometrial polyps in obs.</td>
<td>0.62</td>
<td>0.22 – 1.77</td>
</tr>
<tr>
<td>Metrorrhagia</td>
<td>1.30</td>
<td>0.49 – 3.43</td>
</tr>
<tr>
<td>Transvaginal ultrasound score</td>
<td>2.52</td>
<td>1.69 – 3.75</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval; bold value indicated statistical significance.

![ROC Curve](image)
The results from this study have been confirmed that the thickness of endometrium cannot be observed separately from other parameters which have been measured. This was also shown by the other studies where the ultrasound examination of the thickness of endometrium reached 80.5% of sensitivity, but was predicted to be low-specific 61% and with a dramatic decrease with asymptomatic patients [14, 15].

Appleton and Kupesic-Plavsic pointed out the necessity of an endometrial biopsy or curettage in question. The ultrasound examination with colour Doppler enabled a detailed examination of the thickness, structure, and flow through the endometrium prior to intervention. They placed the cut-off of the thickness of endometrium at five mm and each patient was examined separately, depending on a lower or higher cancer risk, which is consistent with the present research [16].

In the study of Hee et al., retrieved material via hysteroscopy after a suspicion of endometrial polyp and histopathology showed benignity in 96.7%, pre-malignancy in 1.1%, and a malignity in 2.2%. This study, abnormal uterine bleeding and post-menopause were the only factors which were determined to be associated with a higher risk of malignancy, with an OR of 5.07 (95% CI, 2.25–11.41) and 3.41 (95% CI, 1.14–10.24), respectively [17]. Most authors agree that with the increase in the thickness of endometrium, the possibility of occurrence of malignancy increases as well, thus they consider a six-mm thickness of endometrium with women who have not had cycle within the last six months or with perimenopausal women to be hyperplasia.

Gianella et al. in their work aimed at discovering the adequate risk score for discovering an endometrial malignant with symptomatic post-menopausal women. They found that the best predictors of endometrial cancer were recurrent vaginal bleeding (OR = 2.96), the presence of hypertension (OR = 2.01) endometrial thickness > eight mm (OR = 1.31), and age > 65 years (OR = 1.11). These variables were used to create a risk-scoring model (RHEA risk-model) for the prediction of intrauterine malignancy, with an area under the curve of 0.878 (95% CI 0.842–0.908; p < 0.01). At the best cut-off value (score ≥ 4), sensitivity and specificity were 87.5% and 80.1%, respectively [18].

Ultrasound has been necessary so far and all the possibilities of new technologies should be used in order to create a system for detecting patients with a potential malignant disease [19]. In the present sample of perimenopausal women, a transvaginal ultrasound examination was performed in each patient and each parameter of the ultrasound score was evaluated in order to receive a certain score. Regression analysis showed that this transvaginal ultrasound score has independent prognostic values for detection of endometrial malignancy. In the group of women with benign histopathological findings, the average value of this score was 6.96 ± 1.85 (range 4–11), while in the group of women with malignant histopathological findings the average value of this score was 10.38 ± 1.86 (range 7–13). The best validity of transvaginal ultrasound score for detection of endometrial malignancy is observed with a value of 8, specifying the sensitivity of 0.857 and specificity of 0.785. This finding pointed out that females with transvaginal ultrasound score more than 8 should be promptly submitted to an invasive diagnostics of fractional explo- rative curettage or hysteroscopy, while those with a score lower than 8 should be carefully followed and monitored. It has been well-recognised that there is no reliable tests or ultrasound indicator which could diagnose a pre-malignant or initial malignant change on the endometrium. It is often difficult to differentiate a benign change on the endometrium from a malignant and a gynaecologist requires assistance to triage patients. Incorporation of this transvaginal ultrasound score as a screening tool in detection of endometrial malignancy should reduce the number of unnecessary interventions. Angioli et al. have also shown that a score is needed to triage such patients, by publishing Risk of Endometrial Malignant (REM) test by combining serum markers, clinical characteristics, and ultrasound values [20]. Their results, as in the present study, showed that such scoring systems are useful for triage of patients, reduction of the costs of diagnostics and treatment, and for more rapid diagnostics [20].

Kurjakand et al. monitored endometrial volume and Doppler criteria for malignancy on endometrium, including sub-endometrial halo, compactness, existence of intra-cavitary liquid, and neo-vascularisation, and have found that the volume with endometrial cancer amounts to 37.0 ± 31.8 ml, the volume with hyperplasia has medium value 7.82 ml, and that the volume with endometrial polyp is significantly high (mean 2.63 ± 2.12 ml) [21]. There are many studies in literature in which the development of nomograms led to successful applications in oncology and developing an accurate predictive nomogram of malignancy risk would be of great importance, helping to avoid under-treatment of patients with endometrial cancer [22]. The introduction of such a scoring system for predicting endometrial cancer, further research, and usage of technological advancements in the world of cancer diagnostics are still necessary.

Conclusion

Ultrasound in gynaecology is an entity which is developing, is accepting new challenges in examining gynaecological pathologies via new methods and modalities. The present study suggests that transvaginal ultrasound score could be applied as a prognostic marker for detection of endometrial cancer in perimenopausal women. These instruments, in addition to traditional measures of clinical outcome, provide additional information and could be a
part of a more comprehensive prediction of patient’s prognosis. However, the authors would like to emphasize that, at present, these results are not generalizable and further studies with external validation are mandatory.

References


Introduction

Group B Streptococcus (GBS) can cause a variety of diseases; it is a facultative anaerobic streptococcus, because it can also cause cows to suffer from the mastitis, thus it is also known as Streptococcus agalactiae. GBS infection was considered as the major cause of neonatal pneumonia, sepsis, and death in Western societies since 1970s [1].

In recent years, certain study have found that GBS is a very important perinatal pathogen, which could infect the uterus and fetal membranes mainly through the ascending-spreading along the parturient canal, thus causing an intrauterine infection and fetal asphyctic death, as well as chorioamnionitis, endometritis, and urinary tract infections, etc [2]. Meanwhile, it is also the pathogen in neonatal bacteremia, sepsis, pneumonia, and meningitis; the ankyrins on the GBS surface can adhere to the epithelium and endothelial cells, thus causing infections, and death cases of neonatal meningitis, which is through the key step of BBB permeation [3], often occurring in preterm children [4]. Western countries have given great importance to it for a long time. Edmond et al. [5] performed a meta-analysis and revealed that the average incidence rate of live births within three months was 0.053%, with an average mortality rate of 9.660%. USA Centers for Disease Control and Prevention (CDC) had developed the GBS screening and treatment guidelines, which has considerably reduced the incidence and hazards of perinatal GBS. According to the report, about 10% -30% pregnant women carried or were infected by this vaginal bacteria, and about half would transmit it to the newborn during childbirth, leading to the early (within seven days of birth) or late (seven days after birth) invasive infection. Breast milk contamination-caused late-onset infection cases are gradually increased [6]. The early invasive infections are mainly sepsis, meningitis, and pneumonia, with the mortality rate as about 5%. GBS infection could also cause premature rupture of membrane and amniotic infection. A study [7] reported that the PROM incidence of urinary GBS-positive pregnant women was 35%, and the incidence of chorioamnionitis and endometritis was about 21%. In addition, the GBS infections could also cause preterm birth, low-weight birth and very low-weight birth. Schrag et al. [8] reported that the common pathogen of early onset sepsis (EOS) of premature infants was still GBS. Scholars in Chinese Taiwan region believed that after giving antibiotics to prevent GBS prenatally, the early-onset GBS sepsis would significantly decrease [9]. In 2008, Barcaite et al. analyzed 31 literatures published from 1996-2006, which included 24,093 women from 13 countries, and found that the germ-carrying rate ranged from about 6.5% to 36% [10]. According to the report of USA CDC, there was on average 8,000 newborns that were infected by GBS annually, and the mortality was about 5% [11]. The domestic awareness on the hazards of perinatal GBS infection towards the mothers and children is relatively low. In recent years, there gradually appeared several GBS-cause serious infections in the mothers and children, suggesting that GBS causes serious harm and
cannot be ignored. Recently, some domestic death cases caused by the GBS infection were reported. Deng et al. [12] performed the GBS detection towards 234 cases of paraffin specimens of neonatal lung tissues that died of pneumonia in Beijing Children’s Hospital and the detection rate was 65%. The results showed that among the cases of newborn pneumonia death, GBS was first place of pathogens. The GBS carrying rate varies with races, geography, and ages. According to the statistics, vaginal germ-carrying rate of pregnant women in Beijing was about 13%. The rectal and vaginal germ-carrying rate of Chinese pregnant women was about 5% to 20%, which could be transient, intermittent, and chronic. The pregnant women that carried the germ could cause much more serious complications towards the mothers and children and GBS was the most common neonatal infection pathogen in the developed countries [13]. Based on the GBS infection-related symptoms in pregnant women and newborns, as well as the severity of diseases, prenatal GBS screening was particularly important. The spread during birth was the main way of neonatal GBS infection. GBS-positive patients could be given an antibiotic prophylaxis, which could effectively reduce the neonatal early invasive infections triggered by thevertical transmission of GBS.

Currently, the GBS detection methods include the culture method and the real-time PCR method. The advantages and disadvantages of the former: it is easily performed, while time-consuming, requires more than 48 hours, the culture difficulties are high, it might be affected by many factors, especially by antibiotics, and the sensitivity is low. Real-time PCR technology refers to the addition of fluorophores into the PCR reaction system, then to the accumulation of fluorescent signals that is used to monitor the process of real-time PCR, and finally the template is analyzed by the amplification curve. With the emergence of real-time PCR technology and relevant PCR instrument, the conventional distal-end method used for the gene detection in the past is completely changed. Real-time PCR technology could rapidly and reliably perform GBS screening. This study used this technology to detect the GBS situations in pregnant women at 34-37 gestational weeks, aiming to understand their germ-carrying situations in the third trimester in Chengdu, as well as its impacts on the mothers and children.

Materials and Methods

Specimen collection

The study included 1,540 pregnant women, aged 21-45 years old, at 34-37 gestational weeks, and insisted on regular check-ups in the Obstetric Department of Sichuan Provincial People’s Hospital during pregnancy. Vaginal swab: firstly, the excessive secretions were wiped from the genital tract, one sterile polyester swab was then placed in the one-third inferior segment of the genital tract to gently rotate and take the secretions along the genital tract wall, then placed back into a 2.0 pml preservation solution containing sterile swab casing, sealed for detection submission. Anal swab: the swab was carefully inserted into the anus at a depth of at least two to five cm above the anal sphincter, gently rotated along the intestinal wall to obtain the specimens, then placed back into the 2.0-ml preservation solution containing sterile swab casing, and sealed for the detection submission. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Sichuan Provincial People’s Hospital. Written informed consent was also obtained from all participants.

Preparation of nucleic acid

The vaginal and rectal secretions were fully shaken, then squeezed the cotton swab, and drew 1.5 ml liquid into the centrifuge tube for centrifugation at 12,000 r/min for five minutes. The supernatant was then discarded, the precipitate was then added to 1.5 ml sterile saline and shaken evenly, followed by centrifugation at 12,000 r/min for five minutes. The supernatant was discarded, the precipitate was then washed three times, and re-suspended with 50 ul saline, then the extract solid content was added and high-speed vortexing was performed for five minutes, followed by instantaneous centrifugation, for ten minutes at 95°C heating, then ten minutes ice-bathed immediately and centrifugation at 12,000 r/min for another two minutes. The supernatant was then restored for the future detection.

Real-time PCR

Five-ul of the above supernatant was added to the prepared PCR reagents for PCR amplification reaction. The amplification parameters were 37°C for two minutes, 94°C for two minutes, 94°C for 20 seconds, and 55°C for 45 seconds, for 40 cycles.

Results analysis the condition settings

The setting principles of the threshold values of the GBS detection fluorescein FAM and the internal reference fluorescein Texas Red were the following: the thresholds were just above the highest point of the amplification curve of normal negative control (random noise threshold), and the Ct values, which were automatically analyzed and calculated by the instrument and shown in the Reporter window, were recorded.

GBS negative (below the detection limit): FAM Ct value = 40, Texas Red (internal reference) Ct value < 40, and there was a good logarithmic growth curve.

GBS positive: FAM Ct value ≤ 33, and there was a good logarithmic growth curve, reference Ct value (Texas Red) ≤ 40.

Invalid reaction: FAM Ct = 40, and Texas Red (internal reference) Ct value = 40, was re-determined.

Experimental gray zone: FAM 33 < Ct value < 40, caused by systematic or human uncertain factors, would normally lead to a repeat of the test twice for confirmation.

Quality control

Negative control GBS (FAM) Ct value = 40, reference (Texas Red) Ct value < 40, and there was a good logarithmic growth curve. Positive control GBS (FAM) Ct value < 33, and there was a good logarithmic growth curve; internal reference (Texas Red) Ct value ≤ 40.

Statistical analysis

The SPSS13.0 software was used for the statistical analysis, the χ² test was used, with p < 0.05 considered as the statistical significance.
Clinical significance of group B streptococcus testing in late pregnancy

Table 1. — *Comparison of GBS-DNA positive and negative towards the mothers and children [n(%)].*

<table>
<thead>
<tr>
<th>Kind of distress</th>
<th>Cases</th>
<th>Preterm birth</th>
<th>Miscarriage</th>
<th>PROM</th>
<th>Fetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBS-positive</td>
<td>86</td>
<td>25 (29.1%)</td>
<td>17 (19.8%)</td>
<td>23 (26.7%)</td>
<td>21 (24.4%)</td>
</tr>
<tr>
<td>GBS-negative</td>
<td>1454</td>
<td>194 (13.3%)</td>
<td>60 (4.1%)</td>
<td>203 (14.0%)</td>
<td>152 (10.5%)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 8.33 \]

\[ p < 0.005 \]

\[ < 0.005 < 0.005 < 0.05 < 0.005 \]

Results

The GBS-carrying rate detected in the perinatal pregnant women by the real-time PCR method was 5.6% (86/1540) (Table 1).

The preterm birth rate of GBS-positive pregnant women was 29.1% (25/86), and that of GBS-negative pregnant women was 13.3% (194/1454); the comparison exhibited a statistical significance of \( p < 0.05 \).

The miscarriage rate of GBS-positive pregnant women was 19.8% (17/86), and the comparison with that of the GBS-negative pregnant women (4.1%, 60/1454) revealed a statistical significance of \( p < 0.05 \).

The PROM rate of GBS-positive pregnant women was 26.7% (23/86), and the comparison with that of the GBS-negative pregnant women (14.0%, 203/1454) revealed a statistical significance of \( p < 0.05 \).

The fetal distress rate of GBS-positive pregnant women was 24.4% (21/86), and the comparison with that of the GBS-negative pregnant women (10.5%, 152/1454) revealed a statistical significance of \( p < 0.05 \).

Discussion

GBS is the conditioned pathogen that parasitizes in the human inferior digestive tract and urogenital tract. CDC (USA) specifically developed the GBS screening and treatment guidelines [14]. The literature reports a GBS infection rate from 5% to 35% [10, 15]. The clinical data of Thailand showed that between 1996 and 2001, the incidence of GBS infection decreased significantly, while the mortality rate still remained high and that 40% of early-onset children might die from this [16].

Different domestic and international studies have shown that the germ-carrying rates of pregnant women in late pregnancy varied in different regions. In Shanghai, the GBS-carrying rats in the third trimester was 3.7%, while in Beijing, it was 9.2%, and this study investigated 380 cases of pregnant women, and reported a GBS infection rate of 6.8%, close to that of Henan Province (6.3%). Among the 1,540 pregnant women in this study, the GBS-carrying rate was 5.6%, which might be closely related to the irregular application of antibiotics. Shanghai’s living level and economic development is better in China, belong to the first-line city, therefore the density of its resident population towards medical intervention acceptance and antibiotics application was higher, and it could be an important factor that led to the lower detection rate. In addition, the populations with better living standards and economic conditions would have better personal hygiene habits that could also be an important factor that could reduce the detection rate. In such developed countries as USA that have much more standard antibiotics application, the GBS detection rate is relatively higher. Of course, the germ-carrying rate varies according to race, geography, and age, and is also affected by many factors such as gynecological inflammation, detection method, frequency, etc., and might also be related to the study’s sample size. The sampling in late pregnancy could more accurately assess the situation [17].

In recent years, Western countries have conducted much research in GBS, and most scholars believe that is obviously related to the preterm birth, PROM, miscarriage, fetal distress, puerperal infection, neonatal pneumonia, and the neonatal mortality could be as high as 20% to 50% [18].

Previous study [19] considered that the GBS infection was one of the most important causes of premature birth, through the stimulation of intrauterine infection-released inflammatory mediators, such as the interleukin (IL) IL-1, IL-6, IL-8, IL-12, and other cytokines, as well as the phospholipase A and prostaglandin, which promote uterine contractions and caused the premature birth. PROM might also indirectly lead to the premature birth. In the present study, the premature birth rate of GBS-positive pregnant women was 29.1% (25/86), significantly higher than that of GBS-negative pregnant women (13.3%, 194/1454). However it should be noted that the current conventional treatments all prompted that GBS might easily lead to PROM, intrauterine infection, even neonatal sepsis, thus causing serious consequences towards the mothers and children, so that some medical institutions would perform an over-medical intervention in GBS-positive pregnant women, which might be one reason of high preterm birth rate in GBS-positive pregnant women.

It had been previously confirmed that the PROM rate of the patients with urogenital tract-GBS carrying was higher than those non-carriers, and in the PROM patients, the GBS positive rate was significantly higher than the normal pregnant women [12]. The infection is a major pathogenetic factor of PROM, among various pathogens that could cause infection such as Escherichia coli, mycoplasma urealytium, and GBS, etc. GBS exhibits the strongest adsorption and penetration abilities towards the chorion, therefore its complications are among the worst. Pregnant women that carry GBS would be prone to the occurrence of ascending infection, the direct invasion of proteolytic enzymes produced by the retrograde bacteria, combined with the phagocytosis of inflammatory cells produced by the stimulation of bacterial infections towards the body that would reduce the local tension of fetal membranes, leading to PROM. The
study [20] found that the PROM rate of GBS-positive parturients was higher than the negative ones, while among the parturients with PROM, the GBS-positive rate was higher than the normal ones. Another study reported that among 2,745 cases, the PROM rate of GBS-bacteriuria patients was 35%, while that of the non-bacteriuria patients was only 15%. Among the 60 PROM cases, 15 cases were found as the GBS positive, accounting for 25%, among which the cervical germ-carriers accounted for 53%, the vaginal germ-carriers accounted for 73%, and the anal germ-carriers accounted for 100% [21].

In this study, the PROM incidence of GBS-positive pregnant women was 26.7% (23/86), while that of the negative pregnant women was 14.0% (203/1454), the difference was statistically significant, therefore the PROM rate of perinatal GBS-positive women was significantly higher than the negative ones. In addition, the rates of abortion and fetal distress of GBS-positive pregnant women were also significantly higher than the GBS-negative cases, and the difference was statistically significance. Shi et al. [20] detected the rates of cesarean section and fetal distress in the GBS-positive pregnant women that were also significantly higher than the GBS-negative ones, and the difference was statistically significant ($p < 0.05$).

In summary, GBS infections could seriously affect maternal, fetal, and newborn health and positive and effective prevention and treatment measures would be important to reduce the incidence.

While in the clinical practices, the preventive measures are not widely applied, more than 90% GBS-DNA-positive pregnant women in this study were not subjected to prenatal and intrapartum preventive measures, but only administered ampicillin and clindamycin for postpartum infection prevention. Therefore, clinical practices should extend preventive measures to prenatal GBS-positive patients: firstly, the clinics should increase the screening efforts towards the advanced maternal and neonatal GBS infections, while continuously improving the specificity and sensitivity of detection methods, thus the GBS detection rate could be improved. In this study, the real-time PCR method was used as a supplement to the traditional bacterial culture method, and became a rapid, sensitive, and specific method towards prenatal GBS screening; thus it was worthy of the clinical application. Secondly, in order to reduce the serious harm caused by the GBS infections, the women at childbearing age should adopt good personal hygiene habits, maintaining the vulva clean, and timely treating gynecological inflammations, while reducing the chances of infection. In addition, in order to avoid the spread caused by healthcare workers when in contact with the fetus, they should prepare their own personal hygiene, and carry out health education and psychological care towards the GBS-positive patients before birth, and timely identify the high-risk GBS-positive patients, eliminate their doubts in using antimicrobial drugs during pregnancy, and timely prenatal and postnatal interventions should be well-performed.

In short, GBS is a common pathogen that could seriously threaten the health of both mothers and infants. It is very important for the pregnant women to perform vaginal and (or) rectal screening at 34 to 37 gestational weeks; the European and American countries have been already widely carried out this screening, and are achieving good results. The experience of advanced foreign countries provided the present authors with good reference, the comparison of GBS-carrying screening inside the suitable candidates between the present country and foreign countries was an important research topic. The fluorescent quantitative PCR is considered fast, highly accurate, and highly sensitive towards GBS detection [22]. The GBS-carrying rates in the pregnant women varied greatly in different foreign regions, for example it was 6.5%–36.0% in the European countries [23], 2% to 29% in USA, and 13% in Korea [22].

References


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Introduction

Preterm birth is still the leading cause of neonatal morbidity and mortality besides advancement in prenatal care [1]. The etiology of preterm birth is not exactly known, but there are possible factors that may result in it like maternal infection, inflammation, multiple gestation, and placental insufficiency. Independent from the etiological factors, once the pathway begins, the final stage results in a shortened and a dilated cervix. In cases with a shortened and a dilated cervix, mechanical closure of the cervix may be the only hope for delay of delivery to a viable fetal period.

Cervical cerclage procedure has been introduced to prolong pregnancy for a period of time for the newborn to survive [2]. Cerclage procedure can be performed mainly due to three indications: 1) elective cerclage, 2) ultrasound indicated, and 3) emergency cerclage [3].

One of the main pitfalls during emergency cerclage procedure is the difficulty to perform it with protruding membranes. In order to decrease the pressure exerted upon the external cervical ostium, amnioreduction has been discussed in the literature [4-6].

In the present study, the authors aimed to investigate whether amnioreduction has any impact on emergency cervical cerclage outcome.

Materials and Methods

This retrospective study examined the data of women who underwent emergency cervical cerclage for advanced cervical dilatation and protruding membranes and delivered in an obstetric unit of a tertiary center at Kocaeli University School of Medicine, Kocaeli, Turkey, between June 2008 and February 2013. The local ethics committee approved the study.

In this study, the primary outcome measure was to determine whether amnioreduction has any impact on emergency cervical cerclage outcome. Secondary outcomes were gestational weeks at delivery and neonatal outcomes.

Criteria for enrollment included all the pregnant women with a single live fetus who underwent an emergency cervical cerclage procedure performed for advanced cervical dilatation and protruding membranes between 12-28 weeks of gestation. Advanced cervical dilatation and protruding membranes were defined according to sterile speculum examination and transabdominal ultrasound examination. A dilatation ≥ three cm with visible protrusion of intact fetal membranes at or below the external cervical os was considered as advanced cervical dilatation. The cases with amniotic membranes protruding until or beyond the level of bladder outlet on sagittal transabdominal ultrasound examination were considered to be advanced ultrasonographically. Gestational age was determined by the last menstrual period and/or by first trimester ultrasonography if the patient was unsure of the date of her last menstrual period.

Exclusion criteria included fetus with multiple gestations, multiple anomalies, uterine abnormality, preterm premature rupture of
membranes (PPROM), and clinical signs of chorioamnionitis (uterine tenderness, maternal temperature >38°C, fetal tachycardia, WBC count >16,000, CRP >1.5 mg/dl, and foul vaginal smell).

The study population consisted of a total of 84 consecutive pregnant women with advanced cervical dilatation and protruding membranes who had been offered amnioreduction procedure. After patients were evaluated according to inclusion and exclusion criteria, 61 of the 84 patients were analyzed. Among 61 patients who underwent emergency cerclage procedure, 30 patients who accepted amnioreduction formed group 1 and 31 patients who did not accept amnioreduction formed group 2.

According to exclusion criteria, six women with multiple gestations, one fetus with multiple anomalies, one woman with an uterine abnormality, six women with PPROM, and nine fetuses with clinical signs of chorioamnionitis (uterine tenderness, maternal temperature >38°C, fetal tachycardia, WBC count >16,000, CRP >1.5 mg/dl and foul vaginal smell) were excluded from the study.

For the patients who accepted amnioreduction procedure, an ultrasound guided abdominal amnioreduction (ten ml/week) was performed with a 16-gauge amniocentesis needle after adequate sterilization one hour before the operation. After the procedure, intraamniotic infusion of one gram sulbactam-ampicillin was applied. Besides laboratory examinations performed for both of the groups in order to rule out chorioamnionitis, the amniotic material obtained in group 1 was also investigated for gram’s stain, glucose measurement, and microbial culture. Bacterial visualization during gram stain and glucose concentration of < ten mg/dl were accepted as evidence of chorioamnionitis and were not performed cerclage procedure. Three patients in group 1 did not undergo cerclage procedure after diagnosis of chorioamnionitis in the amniocentesis material.

All cerclage procedures were of the McDonald type by the same operator (EC) with Mersilene tape was used as suture placed at the most distal part of the cervix as previously described [7]. All patients were treated according to the same protocol in the postoperative period: hospitalization was maintained and the women were restricted to bed rest for 48 hours. They were discharged at least one week after the operation from the hospital whenever they did not have regular uterine contractions. Daily ultrasound follow-up of amniotic fluid level and vaginal speculum examination for amniotic fluid flow were performed until the patients were discharged from the hospital. Patients had transvaginal ultrasoundography performed weekly after discharge and bed rest was advised until delivery. All the patients received sulbactam and ampicillin (three g/day for seven days, po), amikacin (1.5 g/day for seven days, i.v.), metronidazole (1,000 mg/day for seven days, i.v.), povidone iodine (0.2 g/day for seven days, intravaginal), indomethacin (300 mg/day for three days, rectally) and progesterone (50 mg/day i.m. for ten days).

The statistical analysis of the data was performed using the Statistical Package for Social Sciences. Results were reported as mean ± standard deviation and percentages. Differences between the groups were assessed using chi-square test for categorical data. In order to detect the differences of continuous variables between the groups, Chi-square test was used. For all comparisons \( p < 0.05 \) was considered statistically significant.

### Results

During the study interval, a total of 56 women were eligible for analysis of the study (in one patient from each group, the membranes ruptured during the operation). Selected maternal variables according to the groups are presented in Table 1. Maternal age, primigravidity, previous abortion ≥ one, number of first and second trimester miscarriages, and number of previous preterm deliveries at 24-34 weeks of gestation were similar between the groups. Gestational age and cervical dilatation at admission were also similar (21.3 ± 3.3 vs. 20.6 ± 3.1 weeks; \( p = 0.44 \)).

Mean white blood cell counts and C-reactive protein levels were not statistically significantly different in between the

### Table 1. — Maternal variables according to data of cerclage procedure women who underwent (group 1) and who did not undergo amnioreduction (group 2) [values are n, mean ±standard deviation or n/N (%)].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n=26)</th>
<th>Group 2 (n=30)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at admission (weeks+days)</td>
<td>21.3±3.3</td>
<td>20.6±3.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>28.2±5.3</td>
<td>29.3±5.1</td>
<td>0.42</td>
</tr>
<tr>
<td>Primigravidaity</td>
<td>14 (53.8)</td>
<td>15 (50.0)</td>
<td>0.79</td>
</tr>
<tr>
<td>Previous abortion ≥1</td>
<td>3 (11.5)</td>
<td>3 (10.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>First trimester miscarriage</td>
<td>6 (23.1)</td>
<td>5 (16.7)</td>
<td>0.54</td>
</tr>
<tr>
<td>Second trimester miscarriage</td>
<td>3 (11.5)</td>
<td>4 (13.3)</td>
<td>0.84</td>
</tr>
<tr>
<td>Previous preterm delivery at 24-34 weeks ≥1</td>
<td>2 (7.7)</td>
<td>2 (6.7)</td>
<td>0.54</td>
</tr>
<tr>
<td>Cervical dilatation at admission (cm)</td>
<td>5.0±2.8</td>
<td>4.0±2.2</td>
<td>0.18</td>
</tr>
<tr>
<td>CRP on admission (mg/dl)</td>
<td>2.2±3.2</td>
<td>1.5±1.6</td>
<td>0.33</td>
</tr>
<tr>
<td>WBCa count on admission (µl)</td>
<td>13903±3297</td>
<td>13103±3772</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* C-reactive protein; a white blood cell count.

### Table 2. — Pregnancy outcome among women who underwent (group 1) and who did not undergo amnioreduction (group 2) [values are n, mean ±standard deviation or n/N (%)].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n=26)</th>
<th>Group 2 (n=30)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at cerclage placement (weeks+days)</td>
<td>21.3±3.3</td>
<td>20.6±3.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Operation time (min.)</td>
<td>80.2±16.9</td>
<td>78.3±14.9</td>
<td>0.66</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>28.3±6.1</td>
<td>28.1±5.6</td>
<td>0.74</td>
</tr>
<tr>
<td>Prolongation of pregnancy (days)</td>
<td>53.7±46.1</td>
<td>47.3±36.7</td>
<td>0.56</td>
</tr>
<tr>
<td>Miscarriage (n)</td>
<td>7 (26.9)</td>
<td>9 (30.0)</td>
<td>0.80</td>
</tr>
<tr>
<td>Delivery at 24-28 weeks of gestation (n)</td>
<td>6 (26.7)</td>
<td>5 (23.1)</td>
<td>0.54</td>
</tr>
<tr>
<td>Delivery at 28-32 weeks of gestation (n)</td>
<td>3 (26.7)</td>
<td>5 (26.9)</td>
<td>0.58</td>
</tr>
<tr>
<td>Delivery at 32-34 weeks of gestation (n)</td>
<td>3 (16.7)</td>
<td>7 (11.5)</td>
<td>0.25</td>
</tr>
<tr>
<td>Delivery at 34-37 weeks of gestation (n)</td>
<td>5 (10.0)</td>
<td>3 (15.4)</td>
<td>0.33</td>
</tr>
<tr>
<td>Delivery at ≥37 weeks of gestation (n)</td>
<td>2 (3.3)</td>
<td>1 (7.7)</td>
<td>0.47</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>11 (42.3)</td>
<td>13 (43.3)</td>
<td>0.94</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>15 (57.7)</td>
<td>17 (56.7)</td>
<td>0.94</td>
</tr>
</tbody>
</table>
groups.

Pregnancy outcomes among women who were and were not performed amnioreduction according to the groups are demonstrated in Table 2. Gestational age at cerclage, delivery, and prolongation of pregnancy interval were comparable between the groups (21.3 ± 3.3 vs. 20.6 ± 3.1 weeks; \( p = 0.44 \); 28.3 ± 6.1 vs. 28.1 ± 5.6 weeks; \( p = 0.74 \); 53.7 ± 46.1 vs. 47.3 ± 36.7 days; \( p = 0.56 \), respectively). Also, when the operation time was compared, there was no difference (80.2 ± 16.9 vs. 78.3 ± 14.9; \( p = 0.66 \)). Mis
carriage rates, delivery rates between 24-28 weeks, 28-32 weeks, 32-34 weeks, 34-37 weeks, and >37 weeks were not statistically significantly different between the groups. Mode of delivery was also similar according to the analysis.

Data of newborns according to groups are presented in Table 3. Mean birthweight, neonatal intensive care unit admission (NICU), number of fetuses with birthweight <1,000 grams, 1,000−2,500 grams, and >2,500 grams were similar between the groups. One and five minute Apgar scores were also similar when comparing the groups. Number of live birth rates and perinatal mortality rates were not statistically significantly different between the groups (73.1% vs. 70.0%; \( p = 0.80 \); 15.4% vs. 13.3%; \( p = 0.83 \)). Number of fetuses discharged alive were also comparable in groups one and two (57.7% vs. 56.7%; \( p = 0.94 \)).

**Discussion**

Today, cervical cerclage procedure is still debatable although it being a relatively common operation. The most common accepted indication for cervical cerclage procedure is cervical insufficiency [8]. Protruding membranes usually occur as a consequence of cervical insufficiency. The incidence of cervical insufficiency has been reported to be between 0.05-1% of all pregnancies [9]. Although management of cervical insufficiency is usually problematic, early detection and intervention are more important. However, there is no way to detect insufficiency earlier besides ultrasound findings of cervical length measurements. Therefore, there remains only two choices to manage a patient with protruding membranes during direct visualization: either wait and apply palliative strategies or perform an intervention without detriment to the mother and fetus. In this study, the present results indicated that in almost half of the patients- either with or without amnioreduction- discharge with a healthy fetus could be possible.

Effectiveness of emergency cervical cerclage has been investigated in the literature with regards to interval between delivery and neonatal outcomes [10-12]. Celen et al. have investigated 75 pregnant women with cervical di
latation in the second trimester [10]. They have reported fetal survival rates as high as 89.1% without any compli
cations. Cavus et al. discussed pregnancy outcomes in 20 patients treated with emergency cervical cerclage [11]. They reported 55% of patients with delivery at 36 weeks. Khan et al. compared outcomes of elective, urgent, and emergency cerclage procedures [12]. Their results revealed a mean of 11 weeks of prolongation of pregnancy. The present results indicated 30.0% and 33.6% of deliv
eries after 32 weeks in each group.

Amnioreduction has been introduced for two main purp
oses in the literature: 1) to lower the pressure exerted upon the membranes and 2) to obtain amniotic sample for investigation of intra-amniotic infections [4-6, 13-15]. In 1979, Goodlin introduced amniocentesis in cases with cervi
cal incompetence [4]. Afterwards, Locatelli et al. per
formed amnioreduction of amniotic fluid followed by amnioinfusion and reported higher delivery rates after 32 weeks of gestation [5]. Mays et al. also performed amnioreduction, but this time they investigated amniotic fluid for the presence of chorioamnionitis [6]. Cerqui et al. reported three cases of cervical cerclage with the as
cistance of amnioreduction [13]. Locatelli et al. compared in another study intracervical Foley catheter with am
nioinfusion and suggested lower rates of extreme pre
maturity with amnioreduction [14]. Finally, Makino et al. categorized bulging membranes as type 1 or type 2 ac
cording to protrusion beyond the inlet or completely oc
cupying the vagina [15]. In their study, they also reported similar results in both groups that underwent amnioreduction and cerclage procedure. In the present study, the authors also compared the outcomes in cases with and without amnioreduction. Delivery weeks, birthweights, perinatal mortality rates, and number of discharged alive fetuses were similar in between the groups. In three pa
tients cervical cerclage procedure was going to be per
formed unless amniocentesis materials revealed chorioamnionitis. Hence, although the obstetrical out-
comes are comparable to the patients who did not undergo amnioreduction, the latter may provide investigation of chorioamnionitis from the amniocentesis material.

Main concern regarding the decision to perform emergency cerclage procedure is the possible complications. Kahn et al. reported 17.7% premature rupture of membranes (PRM) and 29.4% uterine contractions until delivery [12]. Liddiard et al. compared elective and emergency cervical cerclage [16]. Opposite to the previous studies, they reported higher complication rates (ruptured membranes in 33% of patients) and lower mean gestation at delivery (26 weeks). The present results indicate a higher gestational age at delivery (28 weeks in both of the groups) with almost no complications including PPROM.

Emergency cerclage was compared with expectant management or bed rest for prolapsed fetal membranes in the literature. Aoki et al. reported comparable prolonged pregnancy duration with emergency cerclage in the absence of signs of infection or painful uterine contractions [17]. Similarly, Stupin et al. compared 89 cerclage cases with 72 conservative procedures retrospectively [18]. In their study, they reported significantly higher live births in the cerclage group (72% vs. 25%; p < 0.001). Daskalakis et al. also compared emergency cervical cerclage with bed rest [19]. Neonatal survival rate was reported as 31% and 94.1%, respectively, between the groups.

Success of emergency cerclage procedure have raised the question of whether outcomes could be predicted. Deb et al. and Guducu et al. analyzed the predictors of success in cases with protruding membranes [20, 21]. In both studies, they concluded that presence of membrane prolapse was a strong predictor factor. Also, Deb et al. made an analysis related with initial WBC and stated a significant association with WBC counts. Gupta et al. also analyzed predictors of success in their study on 45 emergency cerclage patients [22]. They defined chorioamnionitis as predictor of poor outcome. Although not consistent enough, prolapsed membranes, advanced cervical dilation, maternal symptoms, and equivocal markers of infection have been accepted as associated poor outcome markers. Cervical dilation has been compared in the literature as a predictor of pregnancy outcome. While Fortner et al. defined ≥ two cm dilatation for delivery at an earlier gestation, Abo-Yaqoub et al. defined ≥ three cm for cerclage failure [23, 24]. Debby et al. compared emergency second trimester cerclage outcomes in patients with and without bulging membranes into the vagina [25]. They reported favorable outcomes even in cases with bulging membranes.

In conclusion, emergency cerclage yields live take home baby rates in more than half of the patients. The decision to perform amnioreduction should be based on suspicion of chorioamnionitis and patient’s motivation to know exactly what is the risk of chorioamnionitis.

References

Does amnioreduction increase success of emergency cervical cerclage in cases with advanced cervical dilatation and protruding membranes?


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Polymorphisms of p53 promoter and susceptibility to uterine leiomyoma

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Summary

Background: Uterine leiomyomas could be considered as benign tumor of human uterus smooth muscle with unknown etiology and pathophysiology. Furthermore, they are the most common indication of hysterectomy. The tumor suppressor gene p53 has been involved in various malignancies. Mutation in its promoter site may play a role in tumorigenesis of many malignancies including leiomyoma.

Materials and Methods: For study of polymorphisms and allele frequency, 234 female patients with pathologically diagnosed uterine leiomyoma and 100 healthy blood donors as control group were assessed. DNAs were extracted from peripheral blood cells, amplified using polymerase chain reaction and restriction fragment length polymorphism (RFLP) technique was utilized for their analysis.

Result: Proportions of A homozygote/heterozygote/G homozygote for SNP -250 A/G in leiomyoma group were 97.8%, 1.7%, and 0.4%, and in control group 97%, 3%, and 0%, respectively. In case of -216 T/C polymorphism, proportions of T homozygote/heterozygote/C homozygote in leiomyoma were 98%, 1.7%, and 0%, and in control samples 98%, 2%, and 0%, respectively. Genotype frequency of A homozygote/heterozygote/G homozygote for SNP-103 A/G was 97.9%, 1.7%, and 0.4% in leiomyoma group, and 98%, 2%, and 0% in control group, respectively. Proportions of A homozygote/heterozygote/G homozygote for SNP-33 A/G in leiomyoma group were 97.8%, 2.2%, and 0%, and 97%, 3%, and 0% in case samples, respectively.

Discussion: Based on the present results in an Iranian female population, surprisingly there was no significant differences between leiomyoma cases and control samples regarding allele frequencies of p53 promoter polymorphism. Therefore, The p53 promoter polymorphism is not associated with the susceptibility of uterine leiomyomas in Iranian women.

Key words: Uterine leiomyoma; p53; Polymorphism; Iranian women.

Introduction

Uterine leiomyomas are benign clonal tumors of human uterus smooth-muscle cells. They are clinically apparent in about 25% of women [1] and with careful pathological examination of surgical specimens, the prevalence is as high as 77% [2]. Most of women with uterine leiomyomas are asymptomatic, thus often remain undiagnosed [3, 4]. Common signs and symptoms in symptomatic women may include pain, prolonged menstrual periods, bleeding between periods, pelvic or lower back pain, ‘fullness’ in the lower abdomen, with or without urinary or rectal symptoms, due to compression, and reproductive problems, such as infertility, multiple miscarriages, or early onset of labor during pregnancy [5]. The pathophysiology of uterine leiomyomas is not well understood. However, genetic predisposition, as well as steroid hormone concentrations, have a role in formation and growth of these tumors, as do growth factors important in fibrotic processes and angiogenesis [6].

One of the newest and useful means to study the etiology of polygenetic disorders with complex inheritance patterns is the determination of single nucleotide polymorphisms (SNPs) that is used in polygenetic disorders such as diabetes, hypertension, and neoplasms. The phenotypic effects of SNPs are based on direct genetic effects, gene-gene interactions, and gene-environment interactions [7]. Identification and cataloging of SNPs represents a major task for molecular biology in the near future [8]. Polymorphisms are defined as mutations with an allele frequency of at least 1% in a given population [9, 10]. Humans are believed to carry over a million distinct SNPs [10], with around 30,000 of them exerting clinically visible phenotypic effects [7]. Various mechanisms such as enhanced/reduced transcription, altered post-transcriptional or post-translational activities, or changes in the tertiary structure of the gene product may cause these effects [7, 10]. In this regard, the identification of genetic factors that predispose individuals to uterine leiomyoma could provide further insight into the etiology of this benign neoplasm.

As a tumor suppressor protein, p53 function is based on its ability to up- or downregulate the expression of many
genes involved in cell growth, cell cycle progression, DNA repair, cellular senescence, autophagy, metabolism, and p53 regulation [11, 12]. Genomic instability of p53 plays a role in the development and progression of various tumor types, including cervical breast cancer [13], carcinoma [14], esophageal carcinoma [15], lung cancer [16], ovarian carcinoma [17], prostate cancer [18], brain tumor [19], bladder cancer [20], nasopharyngeal carcinoma [21], hepatoma [22], oral carcinoma [23], gastric cancer [24], and lymphoma [25]. Also, some researchers have investigated the undetectable expression of p53 in the leiomyoma specimen [26-30].

Functional inactivation of tumor suppressor genes during tumor progression has been shown to occur by either coding region mutation or promoter region. The segregation, substitution, or deletion within the promoter consequences might directly or indirectly interfere with the guarantee of p53 as well as the consequent tumorigenesis. Transcriptional inactivation of the promoter region may participate in carcinogenesis [31]. Transcriptional repression by p53 promoter methylation might contribute to tumor progression [32]. Therefore, mutations in p53 promoter region might play a partial role in the process of tumorigenesis. Furthermore, the understanding of the detailed characterization of p53 promoter is useful for the elucidation of the underlying regulation of p53 expression.

In a study Hsieh et al. found four SNPs in promoter of p53 protein (-250 A/G, -216 T/C, -103 A/G, and -33 A/G) with higher frequency among other variants in promoter of p53 protein in women with leiomyoma [33].

In this study, the authors attempted to elucidate the relationship between high frequency polymorphisms of p53 promoter gene and leiomyoma. In reviewing the literature, few investigators demonstrated the mutation statuses of p53 promoter as well as its promoter region in leiomyoma individuals [33, 34]. Only one literature revealed the association between the leiomyoma and the p53 promoter genes [33] and there is no current study conducted in Iranian women.

Materials and Methods

In this study, the authors analyzed four highly suspected SNPs in p53 promoter that might be cause of aberrant methylation promoter regions of p53 by the method of restriction fragment length polymorphism (RFLP) in leiomyoma in Iranian women. These SNP included -250 A/G, -216 T/C, -103 A/G, and -33 A/G. In this case-control study, premenopausal Iranian women with pathologically diagnosed leiomyomas and non-leiomyomas were included. Women without leiomyoma were examined by ultrasonography. All women were divided into two groups: (group 1) leiomyoma (n = 234) and (group 2) non-leiomyoma (n = 100). All women did not use hormone therapy in the past one year. Also pregnant women, smokers, postmenopausal women and women with estrogen-related cancers were excluded. After obtaining approval from the ethics committee of Shahrekord University of Medical Sciences, blood samples from all of the individuals were collected. Genomic DNA was extracted from peripheral blood samples with phenol–chloroform extraction method. About 25 ng of genomic DNA was mixed with ten pmole of PCR primers in a total volume of 25 ml containing 10 mM Tris- HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM in each deoxyribonucleotide triphosphate, and one unit of Tag DNA polymerase.

PCR primers were synthesized according to the published p53 GenBank promoter sequence (accession no. X54156). A sequence of the primers were as following: Forward, 5’-GAT CCA AGG GAA GC-3’; Reverse, 5’-CTT ACC CAA TCC GCT GAG AGC AAA CG-3’. The PCR amplification was performed in a PCR machine with the following PCR conditions: one cycle at 95°C for six minutes, 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 40 seconds, and final cycle of extension at 72°C for six minutes. In analyzing by the method of RFLP, the PCR product (464 bp) digestion was performed according to commercial instructions of restriction enzymes. The related enzymes and DNA fragments length are listed in Table 1. Genotypes for p53 promoter polymorphisms in the leiomyoma and control groups were compared.

Table 1. — Restriction enzymes and DNA fragments after digestion for p53 promoter -250 A/G, -216 T/C, -103 A/G, and -33 A/G polymorphisms.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Restriction enzyme</th>
<th>Allele type</th>
<th>DNA fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-250 A/G</td>
<td>Bgl I</td>
<td>WT</td>
<td>464</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>363+101</td>
</tr>
<tr>
<td>-216 T/C</td>
<td>Mae I</td>
<td>WT</td>
<td>355+109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>137+218+109</td>
</tr>
<tr>
<td>-103 A/G</td>
<td>Tau I</td>
<td>WT</td>
<td>464</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>250+214</td>
</tr>
<tr>
<td>-33 A/G</td>
<td>Dde I</td>
<td>WT</td>
<td>464</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>316+148</td>
</tr>
</tbody>
</table>

Table 2. — Genotype distribution of -250 A/G polymorphism of p53 promoter.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leiomyoma n=232 (%)</th>
<th>Non-leiomyoma n=100 (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>227 (97.8)</td>
<td>97 (97)</td>
<td>0.614</td>
</tr>
<tr>
<td>AG</td>
<td>4 (1.7)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Leiomyoma</th>
<th>Non-leiomyoma</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>458 (98.7)</td>
<td>197 (98.5)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>6 (1.3)</td>
<td>3 (1.5)</td>
<td></td>
</tr>
</tbody>
</table>

For statistical analyses, SPSS statistics, version 16.0 was used. Chi-square tests were utilized. A p-value < 0.05 was considered statistically significant.

Results

In this study 234 women with uterine leiomyoma and 100 controls were examined. Mean age in groups were 44.5 ± 5.7 (leiomyoma) and 33.2 ± 9.3 (non-leiomyoma) (p < 0.01). Genotype distribution of different p53 polymorphisms in both groups was not significantly different. Proportions of A homozygote/heterozygote/G homozygote for SNP -250 A/G in leiomyoma group were 97.8%, 1.7%, 0.4%, and in non-leiomyoma group were 97.8%, 1.7%, 0.4%, and in non-
Table 3. — Genotype distribution of SNP -216 T/C polymorphism of p53 promoter.

<table>
<thead>
<tr>
<th></th>
<th>Leiomyoma n=234 (%)</th>
<th>Non-leiomyoma n=100 (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>230 (98)</td>
<td>98 (98)</td>
<td>0.885</td>
</tr>
<tr>
<td>TC</td>
<td>2 (1.7)</td>
<td>4 (2)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>462 (99.6)</td>
<td>198 (99)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2 (0.4)</td>
<td>2 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — Genotype distribution of SNP -103 A/G polymorphism of p53 promoter.

<table>
<thead>
<tr>
<th></th>
<th>Leiomyoma n=234 (%)</th>
<th>Non-leiomyoma n=100 (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>229 (97.9)</td>
<td>98 (98)</td>
<td>0.794</td>
</tr>
<tr>
<td>AG</td>
<td>4 (1.7)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>462 (98.7)</td>
<td>198 (99)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>6 (1.3)</td>
<td>2 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. — Genotype distribution of SNP -33 A/G polymorphism of p53 promoter.

<table>
<thead>
<tr>
<th></th>
<th>Leiomyoma n=230 (%)</th>
<th>Non-leiomyoma n=100 (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>225 (97.8)</td>
<td>97 (97)</td>
<td>0.654</td>
</tr>
<tr>
<td>AG</td>
<td>5 (2.2)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>455 (98.9)</td>
<td>197 (98.5)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>5 (1.1)</td>
<td>3 (1.5)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Human uterine leiomyomas (fibroids) are common benign neoplasms in reproductive age and premenopausal women. These tumors have a significant and increasingly health concern for a large amount women throughout the world. Alternative therapies are few, and the treatment of choice by many physicians is hysterectomy. There is interesting evidence suggesting that the development of leiomyomas may be influenced by many factors [35], including acquired ones such as hypertension, obesity, and early menarche and they may also be associated to genetic changes [36]. Understanding of the genetic tendency for this benign tumor and the specific genes that are dysregulated may indicate new possibilities for pharmaceutical intervention and ultimately lead to strategies for gene therapy and prevention. Primary studies have begun on identification of genes through a genome-wide scan for finding possible mechanisms of gene therapy that take advantage of the physiology of leiomyomas [6, 37].

Aberrant DNA methylation and its related transcriptional aberration were associated with cancer processes, which may show an important primary mechanism that triggers transformation of a single tumor stem cell that will finally develop into a leiomyoma tumor [36]. Understanding the role of apoptosis in the normal regression of myometrial tissue and how its failure may influence on tumorigenesis may help to develop effective and less invasive treatment modalities for this disease [38]. P53 gene and its encoded protein are related with the regulation of cellular growth, cell cycle, and apoptosis. It is a gatekeeper or guardian of cell division [39, 40]. The p53 mutations are associated with instability of cell development and cycle progression [41]. Somatic mutations in p53 gene are the most common genetic alterations found in human malignancies [42] and are detected in approximately half of all cancers. Thus it is reasonable to suppose that genetic changes in the p53 promoter region might determine an individual susceptibility to leiomyoma.

Mutation in promoter regions of the gene is associated with transcriptional inactivation of various tumor suppressor genes in neoplasms [31]. Thus, mutations in p53 promoter region might play a partial role in the process of tumor genesis. Few reports are available on mutations in the p53 promoter in cancers. In contrast, Kullmann et al. demonstrated that analyzing the sequence p53 promoter did not reveal any mutational base change in the rheumatoid arthritis synovial fibroblasts [43]. This indicates that in these patients, p53 mutations in synovial fibroblasts do not contribute to the proliferative and aggressive behavior of these cells. Also Nayak and Das reported the absence of mutations and deletions in p53 promoter in breast tumorigenesis [34]. In fact, the specific mutation pattern of p53 gene appears different expression depending on the types of tissue [43].

The present authors could not find any significant difference between frequency of -250 A/G and -33 A/G, (similar to Hsieh et al.[33]), but also frequency of -216 T/C and -103 A/G appeared with similar distributions between Iranian women with and without leiomyoma. It seems to be in contrast with an analysis in premenopausal Tai-
wane women with surgically diagnosed leiomyoma [33], which suggested that alleles of -216 T/C, -103 A/G within the promoter region of p53 genes were associated with higher susceptibility of leiomyoma development. On the other hand, some studies suggested that the sex steroids influence the growth of leiomyomas by stimulating cell proliferation rather than by affecting apoptosis and no difference in apoptotic index was observed between leiomyomas and normal myometrium [26]. Growth modulation of leiomyomas by hormone deprivation might occur via mechanisms independent of apoptosis [44]. This theory also could confirm the present results.

Conclusion

The present study indicates that p53 promoter polymorphisms including -250 A/G, -216 T/C, -103 A/G, and -33 A/G are not associated with an increasing risk of uterine leiomyoma in Iranian women. The authors suggested sequencing the p53 promoter region in Iranian women with leiomyoma in Iranian women. The authors suggested sequencing the p53 promoter region in Iranian women. In order to detect the novel sequence variations and determine whether mutations in transcription regulatory sequences of p53 gene may result in leiomyoma development or pathogenesis of leiomyoma could be considered a p53 independent and novel study.

Acknowledgments

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Analgesia: effects on the first and second stages of labor

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Summary

Background: This controlled observational study aimed at evaluating the effects of epidural analgesia on the first and second stages of delivery in nulliparous women, referred to the birth centers of the Sant’Omero “Val Vibrata” Hospital and the “San Salvatore” Hospital in L’Aquila, selected in accordance with specific inclusion criteria. Materials and Methods: Between May 1st, 2012 and April 31st, 2013, 363 patients were enrolled at the birth centres of the “Val Vibrata” Hospital in Sant’Omero (TE) and of the “San Salvatore” Hospital in L’Aquila. 139 patients received epidural analgesia during labor at the “Val Vibrata” Hospital; 224 patients constituted the control group and went through natural delivery without analgesia at the “Val Vibrata” and “San Salvatore” hospitals. Results: Dilation time was different in the two groups: in the group with analgesia, the median was 2.30 and 3.35 in the control group. The median expulsion time was 2.05 in the analgesia group and 0.40 in the control group. Discussion: The statistical analysis of the study has highlighted the fact the analgesia influences the dilation and expulsion time of labor, confirming on the one hand the clinical evidence, and on the other, adding important results that have not been analyzed by other scientific studies. The results have shown that in nulliparous women, with spontaneous onset of labor, analgesia causes a major reduction in the dilation time of the cervical canal with respect to the control group.

Key words: Epidural analgesia; First stage of delivery; Second stage of delivery; Labor; Dilation.

Introduction

There is evidence in the scientific literature that pain relief in labor is beneficial for both mother and newborn, and conditions have been identified where analgesia is actually an indication [1]. Maternal benefits include reduction in the consumption of O2 and in hyperventilation which prevents metabolic acidosis [2, 3]. In addition, the greater degree of relaxation and of maternal cooperation cause a decrease in the incretion of catecholamines and of stress hormones, and hence an improvement in blood supply to the placenta [4, 5]. Fetal benefits consist above all in improved neonatal outcomes thanks to the abovementioned benefits on placenta circulation [5, 6]. Over the years many scientific studies have focused on various aspects linked to the use of analgesia during labor, such as neonatal outcome and observation of complications, evaluating differences in the use of subarachnoid, epidural or combined analgesia and the incidence of operative delivery [1, 7, 8]. In the past it was deemed that the latter was related to the use of epidural analgesia [2, 9]. So far there has been ample evidence showing that there is no such relationship [1, 10]. As to the possible effects that epidural analgesia has on the stages of delivery, the literature has shown that in most cases the expulsive phase is prolonged [11, 12]. On the contrary, there are no studies confirming the role of analgesia on the dilation phase of labor [13, 11].

This controlled observational study aimed at evaluating the effects of epidural analgesia on the first and second stages of delivery in nulliparous women, referred to the birth centers of the Sant’Omero “Val Vibrata” Hospital and the “San Salvatore” Hospital in L’Aquila, selected in accordance with specific inclusion criteria.

Materials and Methods

Between May 1st, 2012 and April 31st, 2013, 363 patients were enrolled at the birth centres of the “Val Vibrata” Hospital in Sant’Omero, Teramo, and of the “San Salvatore” Hospital in L’Aquila. The study included 139 patients that received epidural analgesia during labor at the “Val Vibrata” Hospital where an anesthesia service is available 24/7; 224 patients constituted the control group and went through natural delivery without analgesia at the “Val Vibrata” and “San Salvatore” hospitals.

This is a controlled observational study. Nulliparous patients with physiological term pregnancy and spontaneous labor were enrolled. The obstetric criteria for exclusion were: operative delivery, cesarian section, twin pregnancy, obstetric maternal disease (gestational hypertension, endocrinopathy, eclampsia, pre-eclampsia, recent hemorrhage, gestational and pre-gestational diabetes), genetic neonatal anomalies or major malformations, anomalous presentation, macrosomias (neonatal weight greater than 4,500 grams, or > 95th percentile for gestational age), use of maternal prescription drugs, uterine anomalies or pathologies, and positive allergy to local anesthesia, and in addition, patients with excessively inhomogeneous results suggestive of measurement errors or of medical conditions of which the patients were not aware.
The variables taken into account in the study were: maternal age, gestational age, time of dilation of the cervical canal to four cm (moment when the neck of the uterus is centralized and flattened, with the fetal head fixed at least at the upper section, and regular contractions of at least 40 seconds, at which time the first dose of anesthetic is delivered), time of full dilation, time of complete expulsion of the fetus, APGAR scores at one and five minutes after birth, administration of oxytocin, if any, and time when infusion was started, and weight of the newborn.

The anesthetics administration protocol used in the study was the protocol routinely used at the “Fatebenefratelli Villa San Pietro” Hospital in Rome (Table 1).

### Statistical analysis

The analysis was carried out in a total sample of 363 patients enrolled in accordance with the inclusion criteria and the variables examined were maternal age, gestational age, time of dilation of the cervical canal to four cm (first administration of the anesthetic), time of total dilation, time of total expulsion of the fetus, APGAR scores at one and five minutes, use or not of oxytocin, time when oxytocin was delivered, and neonatal weight.

The average, median, and standard deviation values were calculated for each variable followed by a second analysis of the previous evaluations separately in the two groups (group n., patients who did not receive analgesia; group A, patients who received analgesia) (Tables 2, 3, 4). All the variables were subjected to the Wilcoxon test (sum of the ranks) and the Kruskal-Wallis test (evaluation of the chi-square) to confirm the reliability of the data. The frequency and the percentage of oxytocin used by the two groups was then evaluated: 28.78% of the women in group A and 18.75% of the women in group N received at least one dose of oxytocin.

Data consistency was checked by running the Fisher test (Table 5). On the basis of the Spearman correlation coefficient, the most significant variables of group A were: age-expulsion time, age-APGAR score at one minute, age-minutes to expulsion, dilation time-gestational weeks, weight-gestational weeks. In group N the most significant variables were: weight-gestational week, minutes to expulsion-dilation time.

The final analysis with a 95% confidence interval showed that in group N (primiparous women who did not receive analgesia), the odds ratio was 28.78 (median value of expulsion time for all 363 enrolled patients): in group N the probability of having an expulsion time < 1.05 increased by 8.6 times. Expulsion time and dilation time correlated well showing that with a dilation time < 3.10 (median value of the dilation time for all 363 patients enrolled) the probability of having an expulsion time < 1.05 increased by 1.82 times (Table 6).

In group A (women who received analgesia), the odds ratio was 5.6: the probability of a dilation time < 3.10 increased by 5.6 times. Expulsion and dilation times correlated, thus showing that with an expulsion time < 1.05 (median value of the expulsion time...
for all 363 enrolled patients), the probability of having a dilation time < 3.10 increased by 1.8 times (Table 7).

**Results**

In a total of 363 women analyzed, 139 (38.29%) delivered with analgesia and 224 (61.71%) with spontaneous delivery. The results were uniform for maternal age (median 30) and neonatal weight (median 3,263 grams). Dilation time was different in the two groups: in the group with analgesia the median was 2.30 and 3.35 in the control group. The median expulsion time was 2.05 in the analgesia group and 0.40 in the control group. The dilation time therefore lasted more in spontaneous delivery without epidural analgesia, while the expulsion time was higher in deliveries with analgesia.

In the group with analgesia there was a higher proportion of patients requiring the administration of oxytocin (29% vs 19%; p < 0.001).

In group A, the probability of having a dilation time of less than 3.10 (median of the dilation-time variable of the 363 measured samples) increased by 5.71 times (odds ratio gr_cl), while an expulsion time of less than 1.05 (median of
the expulsion time variable of the 363 measured samples) increases the probability of having a dilation time of less than 3.10 (median of the dilation time variable of the 363 measured samples) by 1.81 times.

In group “N” the probability of having an expulsion time of less than 1.05 (median of the expulsion time variable of the 363 measured samples) increases by 8.62 times (odds ratio gr. cl), while the dilatation time of less than 3.10 (median of the dilation time of the 363 measured samples) increases the probability of having an expulsion time of less than 1.05 (median of the expulsion time variable of the 363 measured samples) by 1.81 times.

Discussion

The statistical analysis of this study has highlighted the fact the analgesia influences the dilation and expulsion time of labor, confirming on the one hand the clinical evidence, and on the other, adding important results that have not been analyzed by other scientific studies. Indeed the literature has shown that in nulliparous women with physiological term pregnancy who were treated with analgesia, there was an increase in the duration of the second stage of labor, but there were no statistical evaluations regarding the effects on the first stage of delivery. Hence owing to the poor homogeneity of the samples of patients included and of the various anesthesiology protocols used, the results offered by the literature on this analysis are discordant. The present study intended to minimize such confounding factors by selecting a homogeneous obstetric population, in terms of age, parity, concomitant disorders, neonatal anomalies, and by using a standard anesthesiology technique.

There is extensive scientific evidence showing that analgesia does not cause an increase in operative deliveries and cesarian sections which instead are influenced more by maternal-fetal factors and by obstetric pathologies, and it does not have a role in increasing major neurological incidents.

The aim of the present study was to analyze the influence of analgesia on the first stage of labor since there are little data on this in the literature. At the same time the evaluation was extended to the second stage of labor in order to acquire an overall view of the effects of this anesthesiology technique. The results have shown that in nulliparous women with spontaneous onset of labor, analgesia causes a major reduction in the dilation time of the cervical canal with respect to the control group. In analgesia, the dilation of the cervix is faster for the myorelaxing and sympatholitic effect of the administered drugs. Of course the latter have no effect on the time of descent of the fetus that occurs according to the time of spontaneous delivery without analgesia. This has a twofold advantage: the first is objective and concerns the time gain; the second is subjective and concerns the women in whom the absence of pain enables them not to experience the longer and more painful phase of labor and thus give birth more serenely. On the other hand, failure to perceive pain lowers the perception of the pressing sensation that is normally the input for using pelvic torque that increases the expulsion force exercised voluntarily by the woman. This clinically translates into an increase in expulsion time, a factor that is partially offset by the use of an intravenous infusion of oxytocin in the women who undergo analgesia. On the basis of the present evidence, the authors can state that analgesia accelerates the dilation period of delivery, thus extending the duration of delivery a little and in a non significant manner and it increases the need of the administration of oxytocin without influencing the vitality of the newborn measured as Apgar score at one and five minutes.

Conclusion

In conclusion the present authors’ experience confirms that analgesia during labor is useful, is a safe technique for both mother and newborn, and offers an unquestionable advantage for mothers who can face labor more serenely and with greater emotional participation without interfering in neonatal outcome. Clinically it reduces the cervical dilation time without influencing the delivery mechanism in terms of overall length and incidence of operative deliveries and hence it is recommended as a safe and effective technique for natural delivery.

References


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Elective cervical cerclage versus no treatment in women with the history of cervical insufficiency: retrospective analysis of pregnancy outcomes

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2 Goztepe Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul (Turkey)

Summary
Purpose of investigation: To evaluate the effectiveness of elective cervical cerclage (CC) on the pregnancy outcome of patients with cervical insufficiency. Material and Methods: A retrospective cohort study was conducted on women with an obstetric history of cervical insufficiency on whom CC was applied or not. The two groups were compared for the main measure outcomes of mean gestational age at delivery, birth weight, Apgar scores at five minutes, number of premature and preterm deliveries, rate of preterm premature rupture of membranes, incidence of neonatal death, and admission to the neonatal intensive care unit (NICU). Results: A total of 183 women were eligible for the final analysis in the CC group and 183 were taken as the control group. There were significant differences in terms of the mean gestational age at delivery (37 ± 4.0 vs. 34±5 weeks, p = 0.001), the mean birth weight (3,000 ± 870 vs. 2,200 ± 860 grams, p = 0.001), the number of preterm deliveries (< 37 weeks) (40% vs. 63%, p = 0.001, OR: 0.4, 95% CI: 0.26–0.61) between CC and control groups, respectively. Median Apgar scores at five minutes were 9 in CC group and 8 in the control group (p = 0.001) and the percentages of admission to NICU were 14% in CC group and 34% in the control group (p = 0.001, OR: 0.30, 95% CI: 0.17–0.52). Conclusion: The placement of elective CC seemed to be effective in patients with a history of mid-trimester abortion or preterm delivery due to cervical insufficiency.

Key words: Cervical insufficiency; Cervical cerclage; Pregnancy outcome.

Introduction
Cervical insufficiency is characterized by painless dilation of the cervix in the second trimester. Cervical cerclage (CC) has been performed to prevent preterm delivery in order to decrease the adverse outcomes of cervical insufficiency. Transvaginal CC is applied either electively (prophylactic) depending on obstetric history or selectively (therapeutic) depending on ultrasonographic findings or emergently in the case of advanced cervical dilatation. Many clinicians use serial ultrasound assessment in the management of high-risk women to detect cervical changes prior to preterm delivery and then, selectively place cerclage to women with short cervix. A pregnancy is considered to be at high-risk for cervical insufficiency if the patient had a history of painless cervical dilatation. There are conflicting results whether women with a history of cervical insufficiency should be electively put on cerclage or if they should be followed by serial ultrasound for selective cerclage [1-5].

The aim of the present study was to evaluate the effectiveness of elective CC on the pregnancy outcome of patients with cervical insufficiency.
498 women with a history of mid-trimester abortion or preterm delivery due to cervical insufficiency

104 women with mid-trimester abortion or preterm delivery due to uterine anomaly, history of cervical surgery, polyhydramnios, multiple pregnancy, abruption, iatrogenic early delivery or chorioamnionitis were excluded

211 cases with a history of elective cerclage

183 women without a history of elective cerclage were taken as control

Obstetric records of 28 cases were not reached

183 cases with a history of elective cerclage for final analysis

Figure 1: Flow diagram of the study.

for the main measure outcomes of maternal demographics and past obstetric history, mean gestational age at delivery, birth weight, Apgar score at five minutes, number of premature and preterm deliveries, preterm premature rupture of membranes (PPROM), neonatal death, and admission to the neonatal intensive care unit (NICU).

The CCs were performed by several senior obstetric specialists with McDonald technique at 13-15 weeks of gestation under general anesthesia unless contraindicated. The patients were discharged from the hospital on the following day. In the case of PPROM, the cerclage was removed based on gestational age. Those who had PPROM beyond 32 weeks of gestation or before 22 weeks of gestation, had their cerclages removed. Those who had PPROM between 22 and 32 weeks of gestation, the timing of cerclage removal were individualized. As standard procedure, CCs were removed at 36-37 weeks of gestation. Patients presenting with progressing premature labour prior to 36 weeks of gestation, had their cerclages removed at that time. In general, broad spectrum antibiotics and steroids were administered to the patients with PPROM.

All statistical analysis was performed with the Number Cruncher Statistical System (NCSS) 2007. Normally distributed variables were compared with Student t-test and Mann Whitney U test were used for variables not distributed normally. Qualitative data were analyzed by using Pearson Chi-square test, Fisher’s Exact test, and Yates Continuity Correction test. A p-value of < 0.05 was accepted as statistically significant.

Results

A total of 498 patients, 183 women in the CC group and 183 women in the control group were included in the final analysis (Figure 1). No significant differences were observed between the groups in terms of the mean maternal age, gravidity, parity, body-mass index, the number of previous mid-trimester loss, preterm deliveries (< 37 weeks), term delivery, and alive baby (Table 1).

There were significant differences in terms of the mean gestational age at delivery (37 ± 4.0 vs. 34 ± 5 weeks, p = 0.001), the mean birth weight (3.000 ± 870 vs. 2,200 ± 860 grams, p = 0.001), the number of preterm deliveries (< 37 weeks) (40% vs. 63%, p = 0.001, OR: 0.4, 95% CI: 0.26–0.61), median Apgar scores at five minutes (9 vs. 8, p = 0.001) between CC and control group, respectively (Table 2). No statistically significant differences were found bet-
Table 1. — Demographic characteristics and past obstetric history of the patients in CC and control group.

<table>
<thead>
<tr>
<th></th>
<th>CC group (n=183)</th>
<th>Control group (n=183)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years, mean±sd)</td>
<td>30±6</td>
<td>29±6</td>
<td>0.13</td>
</tr>
<tr>
<td>Gravity (mean±sd)</td>
<td>4±2</td>
<td>4±2</td>
<td>0.74</td>
</tr>
<tr>
<td>Parity (mean±sd)</td>
<td>3±1</td>
<td>2±1</td>
<td>0.10</td>
</tr>
<tr>
<td>BMI (kg/m², mean±sd)</td>
<td>24±3</td>
<td>23±3</td>
<td>0.12</td>
</tr>
<tr>
<td>Mid-trimester abortion (≥1%)</td>
<td>79%</td>
<td>87%</td>
<td>0.10</td>
</tr>
<tr>
<td>Preterm delivery &lt; 37 w (≥1%)</td>
<td>46%</td>
<td>50%</td>
<td>0.56</td>
</tr>
<tr>
<td>Term delivery (≥1%)</td>
<td>18%</td>
<td>26%</td>
<td>0.18</td>
</tr>
<tr>
<td>Alive baby (≥1%)</td>
<td>96%</td>
<td>91%</td>
<td>0.13</td>
</tr>
</tbody>
</table>

CC: cervical cerclage; BMI: body mass index; sd:standard deviation; w:weeks. 
p<0.05 is considered statistically significant.

Table 2. — Comparison of pregnancy outcomes of the patients in the CC and control group.

<table>
<thead>
<tr>
<th></th>
<th>CC group (n=183)</th>
<th>Control group (n=183)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (week, mean±sd)</td>
<td>37±4</td>
<td>34±5</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight (gr,mean±sd)</td>
<td>3000±870</td>
<td>2200±860</td>
<td>0.001</td>
</tr>
<tr>
<td>Apgar scores at five minutes (median)</td>
<td>9</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>PPROM (n, %)</td>
<td>24 (13%)</td>
<td>39 (21%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Neonatal death (n, %)</td>
<td>11 (6%)</td>
<td>20 (11%)</td>
<td>0.13</td>
</tr>
<tr>
<td>NICU (n, %)</td>
<td>25 (14%)</td>
<td>63 (34%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Number of deliveries at (n, %)

<table>
<thead>
<tr>
<th></th>
<th>CC group (n=183)</th>
<th>Control group (n=183)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20w or &lt;500gr</td>
<td>14 (8%)</td>
<td>11 (6%)</td>
<td>0.68</td>
</tr>
<tr>
<td>20-23+6w</td>
<td>4 (2%)</td>
<td>5 (3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>24-27+6w</td>
<td>7 (4%)</td>
<td>8 (4%)</td>
<td>1.00</td>
</tr>
<tr>
<td>28-31+6w</td>
<td>9 (5%)</td>
<td>25 (14%)</td>
<td>0.007</td>
</tr>
<tr>
<td>32-36+6w</td>
<td>40 (22%)</td>
<td>66 (36%)</td>
<td>0.003</td>
</tr>
<tr>
<td>≥37w</td>
<td>109 (60%)</td>
<td>68 (37%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CC: cervical cerclage; NICU: neonatal intensive care unit; sd: standard deviation; gr: gram; w: weeks.

Discussion

In the present study the outcomes of patients with or without elective CC were compared. The results showed significantly better pregnancy outcomes in the elective cerclage group than the control group. The likelihood of developing preterm delivery (< 37 weeks) was lower following the elective cerclage placement (OR: 0.4, 95% CI: 0.26 –0.61) and also, higher mean weight was observed in CC group. Median Apgar scores at five minutes were statistically higher in the cerclage group but this did not mean clinical significance because the median of Apgar scores were eight and nine. The authors also found lower rate of attending to the NICU in the cerclage group which may be related to the lower rate of preterm birth after elective cerclage placement (OR: 0.30, 95% CI: 0.17 - 0.52).

Options for the clinical management of the patients with a history of mid-trimester fetal loss or early preterm delivery are elective cerclage in early second trimester or close cervical surveillance and placement of a CC selectively only if there are cervical changes demonstrated by ultrasound. There are conflicting results about the management of these patients in the current literature [1, 2, 3-5]. Some authors reported that many elective cerclages have been performed unnecessarily and awaiting ultrasound finding of cervical insufficiency prior to placement of a cerclage will result in a decrease in the number of unnecessary elective cerclages [6]. In To et al. study, with the policy of sonographic surveillance followed by CC in women at increased risk of spontaneous mid-trimester or early preterm delivery, the expectant management results in a decrease in the requirement of the cerclage with a rate of 40% but in the elective group the preterm delivery rate was lower than the ultrasound indicated group (15% vs. 31%) [7]. In the present study, the preterm delivery rate was significantly lower in the cerclage group than the control group (40% vs. 63%, respectively), but the early pregnancy loss rates were similar (11% vs. %10). Guzman et al. reported that patients with shortened cervix demonstrated by ultrasound, benefited from the placement of a selective cerclage and they found no significant difference in pregnancy outcomes between elective and selective cerclages [8]. Conversely, Nelson et al. found that selective cerclage placement might have helped prolonging pregnancy and reducing premature birth, but higher rate of obstetrics morbidities such as a higher frequency of PPROM (64.7%) and chorioamnionitis (42.9%) were noted in selective cerclage group [3]. Similarly, Kurup et al. reported that patients who had selective cerclage placement after ultrasound findings of cervical insufficiency had poorer obstetric outcomes than those who had cerclages placed electively [2]. In order to consider sonographic surveillance followed by CC as an alternative to the elective cerclage, pregnancy outcome should be compromised when the selective cerclage is placed after the detection of cervical shortening.

Women with a history of spontaneous mid-trimester abortion and preterm delivery have an increased risk of recurrence in the subsequent pregnancies. According to meta-analysis results of Berghella et al., cerclage does not prevent preterm birth in all women with short cervix, but in the subgroup of women with prior preterm birth, cerclage may reduce premature birth [9]. The recent ACOG Practice Bulletin was published in the favor of elective cerclage that recommends the placement of cerclage at ap-
proximately 13-14 weeks of gestation in women with the history of one or more second-trimester pregnancy losses related to painless cervical dilation and in the recommendation of labor or abruptio placentae [10]. This recommendation supports the effectiveness of elective cerclage and the present study results. In expectant management, it is not easy to follow up the patients closely and to detect high-risk patients early. Guzman et al. described how an incompetent cervix may shorten at a rate of four to eight mm per week between 15 and 24 weeks of gestation [8]. They suggest following high-risk patients with scans every two weeks that allow performing more timely intervention. As described previously, To et al. performed transvaginal sonography in women with a history of one or more mid-trimester miscarriage or early preterm delivery at 12–15+6, 16–19+6 and 20–23+6 weeks [7]. Missing the high-risk patients during the screening of cervical length may cause advanced cervical dilatation and need for emergent cerclage which is associated with a high risk of adverse outcome [11]. Also, improper selection of the patients for the cerclage may lower the benefit. In the present study, there were no statistically significant difference between the groups with regards to adverse outcomes as PPROM and neonatal death, but the incidence of preterm delivery was significantly higher in the control group.

The present results show that the placement of elective CC may be effective in patients with an obstetric history of mid-trimester abortion or preterm delivery due to cervical insufficiency. Although the present results were in the favor of elective cerclage, these findings should be interpreted cautiously. However, these results were the consequence of retrospective analysis. The present study was not randomized, but the similarity in the baseline characteristics of the groups can make it possible to compare the outcomes and control known confounders. The small sample size, the presence of possible bias in the selection of the control group, and possible adverse outcome of the patients whose obstetric outcomes were not reached in the CC group are the limitations of this study.

In conclusion, the placement of elective CC seemed to be effective in patients with the history of mid-trimester abortion or preterm delivery due to cervical insufficiency.

References


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Effects of adding different doses of clonidine to intrathecal bupivacaine for spinal anesthesia in cesarean section

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Summary

Purpose: This study investigated the effects of three different doses of clonidine in combination with intrathecal hyperbaric bupivacaine on the quality of the blockade and maternal-fetal repercussions in parturients undergoing elective cesarean section. Materials and Methods: Following ethics committee approval, ASA I and II patients of age group 20-35 years, scheduled for cesarean section, were chosen for this study. Patients were randomly distributed into three equal groups of 35 patients in each using a computer-generated sequence of numbers. The patients received hyperbaric bupivacaine (two ml) with 15 µg of clonidine (BC15 group) or 30 µg of clonidine (BC30 group) or 60 µg of clonidine (BC60 group). Hemodynamic parameters, onset, peak and duration of sensory and motor block, sedation scores, Apgar scores, side effects, and duration of postoperative analgesia were compared. Results: All groups were comparable with respect to demographic profile, onset, peak and duration of sensory and motor block, and overall hemodynamic stability. The authors observed dose-dependent variability in duration of analgesia and sedation. Duration of analgesia was significantly higher in BC60 group as compared to the other two groups (577.13 ± 120.30 vs. 422.06 ± 112.47 and 376.21 ± 87.21 minutes, respectively). Sedation was also more in BC 60 group. Conclusion: Intrathecal addition of 15 and 30 µg clonidine are better options when sedation is not desirable; on the contrary, addition of 60 µg provides excellent quality of spinal analgesia when some amount of sedation is acceptable or required without any deleterious effects on the mother and baby.

Key words: Spinal anesthesia; Hyperbaric bupivacaine; Clonidine; Cesarean section; Analgesia.

Introduction

Spinal blocks with disposable, low caliber spinal needles and the use of hyperbaric bupivacaine associated with adjuvant became the technique of choice for elective cesarean sections and in urgent and emergency situations [1]. Bupivacaine, a highly lipophilic long-acting local anaesthetic, has been the most commonly used anesthetic agent in these cases. Even when a long acting local anesthetic like bupivacaine is used, the duration of spinal anesthesia (SA) is short and higher doses of analgesics are required in the postoperative period [2]. In order to improve the quality of intraoperative anesthesia, postoperative analgesia and aid early ambulation and recovery of motor block, several adjuvants have been employed such as opioids and α-2 adrenergic agonist like clonidine [3-8]. Some recent placebo-controlled studies suggest that α-2 adrenergic agonist has both analgesic and sedative properties when used as an adjuvant in local anesthesia [8-12].

Intrathecal clonidine is being extensively evaluated as an alternative to neuraxial opioids for control of pain and has proven to be a potent analgesic, free of some of the opioid-related side effects [13]. In clinical studies, addition of clonidine to local anesthetic has been shown to improve peripheral nerve blocks by reducing onset time, improves efficacy of the block during surgery, and prolongs postoperative analgesia [14-18]. Furthermore, it has been reported that clonidine also offers other beneficial effects such as antiemesis, reduced post-spinal shivering, anxiolysis, and sedation [19]. However, its use is limited due to side effect that includes impact on hemodynamic variable. In parturients at term, a higher dose of clonidine may have deleterious effects on fetus, moreover, considering the fact that Chinese population has relatively lower body weight and there is limited research with low-dose clonidine. Thus, it is important to discover the lower effective dose of clonidine to avoid its known side effects like bradycardia, hypotension, and sedation.

The objective of the present study was to assess the effectivity of three different doses of clonidine in combination with hyperbaric bupivacaine in the quality of the blockade and maternal-fetal repercussions in parturients undergoing elective cesarean sections. The study aimed to discover the lowest possible effective dose among the three doses tested. Primary outcome measure compared was duration of effective analgesia measured by time in minutes for requirement of rescue analgesia. Secondary outcome measures compared were demographic characteristics, onset peak and duration of sensory and motor blockade, level of sedation, maternal hemodynamic parameters, and fetal parameters.

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Materials and Methods

This double-blind prospective investigation was conducted from January 2014 to September 2014. Following approval from the institutional review board, written informed consent was obtained from the subjects. A total of 105 patients aged between 20-35 years, assessed as ASA class I or II scheduled to undergo lower segment cesarean section under subarachnoid block, were enrolled double-blind prospective randomized controlled trial. Exclusion criteria included complicated or multiple pregnancy, history of hypersensitivity to clonidine, diabetes mellitus, psychiatric or neurologic diseases, morbid obesity (body mass index > 40), coagulation disorders, infection at the site of injection, and patient’s refusal of regional anesthesia. The demographic profile, medical history, ASA physical status, and allergies of subjects were recorded prior to surgery and all subjects were made familiar with the study plan and visual analogue scales (VAS) to be used in the assessment. Throughout the preoperative period, respiratory rate, arterial blood pressure, peripheral arterial saturation, and heart rate were recorded for all patients. Sedatives and hypnotics were avoided in pre-, intra-, and postoperative periods. All subjects were pre-medicated with an antiemetic agent – inj. ondansetron (four mg intravenously [i.v.]). In addition, Ringer’s lactate solution ten ml/kg was infused to all patients.

The subjects were randomized into three groups of 30 patients each by using a computer-generated sequence of numbers and sealed envelopes were used for allocation. The three groups underwent the following treatments: group 1 (BC15) (n=35): two ml of 0.5% hyperbaric bupivacaine + 15 µg clonidine, group 2 (BC30) (n=35): two ml of 0.5% hyperbaric bupivacaine + 30 µg clonidine, and group 3 (BC60) (n=35): two ml of 0.5% hyperbaric bupivacaine + 60 µg clonidine.

Spinal anesthesia was performed at L3-4 or L4-5 using 26G Quincke’s spinal needle in sitting or lateral decubitus position under all aseptic precautions. Respective agents were injected depending on the groups assigned. The total volume of the drug injected was made up to 3.0 ml in each by adding saline wherever needed. All the drugs were prepared just before the spinal anesthesia by an experienced anesthesiology resident blinded to the study. The assessments of the haemodynamic parameters were noted. Onset of sensory block was tested by pin-prick method every two minutes until T6 level was achieved. Motor blockade was assessed by using modified Bromage scale. An experienced anesthesiologist, who was unaware of the drug administered, evaluated the spinal block and other physiological parameters. Following parameters were observed and recorded: T0 – time of spinal anesthesia, T1 – time of onset of sensory block, T2 – time of onset of motor block, T3 – time of peak sensory block, T4 – time of peak motor block, T5 – time of two segment regression of sensory block, and T6 – time to first dose of postoperative rescue analgesia.

Whenever patient’s VAS score crossed 7, postoperative analgesic drugs were given and this time was considered as the time of wear off of analgesia. Inj. diclofenac 75 mg was given intramuscularly as rescue analgesia.

Side effects such as hypotension (systolic blood pressure less than 20% of the baseline value), bradycardia (heart rate < 50 beats per minute), nausea, pain, vomiting, pruritis, shivering, respiratory discomfort, and sedation were recorded and treated with appropriate drugs if needed. The maternal sedation was recorded using the 4-point ordinal sedation scale (1 = wide awake and alert, 2 = awake but drowsy, responding to verbal stimulus, 3 = arousable, responding to physical stimulus, 4 = not arousable, not responding to physical stimulus) at five, 15, 30, 45, 60, and 90 minutes of drug administration. Umbilical artery blood pH and pO2 were measured at delivery and newborn’s Apgar scores were determined by a pediatrician not otherwise involved in the study at one, five, ten and 15 minutes. Assessment of block characteristics and hemodynamics were the primary outcome and the intraoperative side effects and postoperative pain were secondary outcome measures.

Statistical analysis

One-way analysis of variance (ANOVA) was conducted to analyze continuous variables and Chi-square ($\chi^2$) test was conducted to analyze categorical variables. If the significant group differences were observed, HSD Turkey post hoc test was conducted to assess the differences between individual groups. The data analysis was conducted using SPSS version 20.0. Moreover, descriptive statistics such as mean, standard deviation, and percentage were calculated. A $p$-value less than 0.05 was considered statistically significant.

Results

A total of 105 patients were divided randomly into three groups with 35 patients each were studied. As shown in Table 1, patient characteristics e.g., age, weight, height, ASA physical status, systolic and diastolic BP, duration of pregnancy, and duration of surgery were comparable in all three groups and differences among them were not statistically significant. The baseline systolic and diastolic pressures were normal and are recorded in Table 1.

Block characteristics are presented in Table 2. Results show that the time for onset of sensory blockade and time for onset of motor blockade of the three groups were sta-
Effects of adding different doses of clonidine to intrathecal bupivacaine for spinal anesthesia in cesarean section

statistically comparable and similar, however the BC60 group seemed to reach sensory block slightly earlier than BC30 and BC15, but the difference was not significant on statistical evaluation. The time to onset of motor block was similar in all groups with no significant variation among the three groups. Sensory block reached its peak at an earliest in BC60 group in 6.41 ± 1.19 minutes followed by BC30 in 6.74 ± 1.32 minutes and the latest by BC15 in 7.21 ± 1.47 minutes. There was a significant difference in time taken to reach peak sensory block at p < 0.05 among the three groups. The exact trend was observed in regression time for sensory blockade of the three groups, wherein the maximum regression time was taken by patients in BC60 followed by BC30 and the last by BC15. A statistical significant difference was observed in the regression time with p < 0.05.

Motor block wearing-off time was similar in all the three groups with no significant difference, although patients in BC60 were slower in gaining motor control than other two groups, however this revealed no difference upon statistical evaluation. BC60 group required the least instances of post-operative analgesia than other two groups. The time for first rescue analgesia varied greatly among the three groups. The first rescue analgesia was administered on an average time of 577.13 ± 120.30 minutes in BC60 group, 422.06 ± 112.47 minutes in BC30 group, and 376.21 ± 87.21 minutes in BC15 group. The BC15 group required an earliest rescue analgesia among the three groups. The time for first rescue analgesia showed a statistically significant difference at p < 0.02.

Heart rates were statistically similar throughout the procedure with incidence of bradycardia observed in all groups which was, however, also statistically similar among all the groups. Systolic blood pressure was higher in BC15 group as compared to BC30 and BC60. BC30 and BC60 showed no significant difference in systolic blood pressures. The number of patients with SBP < 20% of baseline was alternatively higher in BC60 (n=4) group and BC 30 (n=4) group as compared to BC15 (n=3). Diastolic blood pressure was higher in BC15 group as compared to BC30 and BC60 group; however, diastolic blood pressure of patients in BC30 and BC60 showed no significant difference (Table 3).

Sedation scores among the three groups did not differ statistically at five and 90 minutes of drug administration (Table 4). At 15 and 30 minutes, BC60 and BC30 groups had more sedation as compared to BC15. A comparison between BC30 and BC60 at 15 and 30 minutes revealed no significant difference although the range of the sedation scores varied between the groups, which proved to be statistically insignificant. At 60 minutes, the sedation scores in BC30 and BC60 were statistically similar in frequency and ranges but altogether significantly different from sedation scores of BC15. The neonatal Apgar scores at one and five minutes, umbilical artery pH, oxygen partial pressure, and carbon dioxide partial pressure were statistically similar in all the three groups (Table 5).

Table 2. — Sensory and motor block characteristics.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BC15 group</th>
<th>BC30 group</th>
<th>BC60 group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time for onset of sensory blockade (minutes)</td>
<td>0.92 ± 0.38</td>
<td>0.91 ± 0.45</td>
<td>0.89 ± 0.46</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Time for onset of motor blockade (minutes)</td>
<td>1.61 ± 0.66</td>
<td>1.49 ± 0.39</td>
<td>1.39 ± 0.47</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Time for peak of sensory blockade (minutes)</td>
<td>7.21 ± 1.47</td>
<td>6.74 ± 1.32</td>
<td>6.41 ± 1.19</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Two segment regression time for sensory blockade (minutes)</td>
<td>122.23 ± 10.87</td>
<td>132.63 ± 13.14</td>
<td>148.14 ± 12.62</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Time for wearing off of motor block (minutes)</td>
<td>174.03 ± 11.14</td>
<td>176.31 ± 11.74</td>
<td>180.01 ± 13.53</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Time for first rescue analgesia (minutes)</td>
<td>376.21 ± 87.21</td>
<td>422.06 ± 112.47</td>
<td>577.13 ± 120.30</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

Note: values are mean ± SD.

Table 3. — Hemodynamic parameters and complications.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BC30 group</th>
<th>BC60 group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>82.8 ± 11.2</td>
<td>81.4 ± 10.8</td>
<td>80.5 ± 10.2</td>
</tr>
<tr>
<td>Bradycardia (&lt; 50 bpm)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115.25 ± 10.87</td>
<td>102.63 ± 13.14</td>
<td>99.14 ± 12.62</td>
</tr>
<tr>
<td>Number of patients with SBP &lt; 20% of baseline (n)</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>65 ± 9.55</td>
<td>57 ± 8.95</td>
<td>54 ± 8.81</td>
</tr>
</tbody>
</table>

Note: values are mean ± SD.

Table 4. — Sedation scores.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>BC15 group</th>
<th>BC30 group</th>
<th>BC60 group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (1-2)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>15</td>
<td>1 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (1-2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>30</td>
<td>1 (1-2)</td>
<td>1 (1-3)</td>
<td>2 (1-2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>60</td>
<td>1 (1-1)</td>
<td>1 (1-3)</td>
<td>1 (1-3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>90</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Scores are median (range).
In the present study, the effects of addition of different doses of clonidine as an adjuvant to bupivacaine were observed to discover the effective lowest dose among them by evaluating the effects versus side effects. During the recent past, clonidine which is a selective partial agonist for α-2 adrenoreceptor has been used to prolong spinal anaesthesia. Various studies have demonstrated that addition of clonidine to bupivacaine significantly improves the onset of both sensory and motor block of local anaesthetics [14, 15, 20]. This action of clonidine is because of spinal cord antinociception via post-junctional α-2 adrenoreceptor mediated noradrenaline release in the dorsal horn [21-23]. Furthermore, clonidine has cholinergic effects and increases the amount of acetylcholine available for modulating analgesia [24].

There were very few incidences of hypotension or bradycardia in all three groups. Only two out of a of total six patients required drug intervention to correct bradycardia and both patients responded well to the atropine. It was discovered that there was no statistical difference between these three groups in terms of bradycardia. Similar findings were reported by previous studies which reported very few incidences of hypotension and bradycardia requiring intervention [14, 15, 20]. It has also been discovered that incidence of hypotension and bradycardia depended on multiple confounding factors such as type of surgery, dose of LA used, hydration status, level of sympathetic block, etc. [14, 20]. Furthermore, previous studies have shown that incidence of both hypotension and bradycardia was more in bupivacaine group than in bupivacaine with clonidine group up to 50 µg [14]. Thus, due to low doses of clonidine and bupivacaine used in this study, there was no significant difference regarding hypotension and bradycardia among the three groups.

Table 5. — Neonatal APGAR scores.

<table>
<thead>
<tr>
<th></th>
<th>BC15 group</th>
<th>BC30 group</th>
<th>BC60 group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APGAR score at one minute</td>
<td>8 (7–10)</td>
<td>8 (7–10)</td>
<td>8 (8–10)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>APGAR score at five minutes</td>
<td>10 (9–10)</td>
<td>10 (9–10)</td>
<td>10 (9–10)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Oxygen partial pressure (mmHg)</td>
<td>20.17 (10–43)</td>
<td>20.13 (11–44)</td>
<td>21.15 (11–36)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Umbilical artery pH</td>
<td>7.2 (0.2)</td>
<td>7.3(0.2)</td>
<td>7.3(0.3)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Carbon dioxide partial pressure (mmHg)</td>
<td>50.13 (37–70)</td>
<td>52.15 (36–71)</td>
<td>50.30 (39–75)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Scores are median (range).
Effects of adding different doses of clonidine to intrathecal bupivacaine for spinal anesthesia in cesarean section

Influence of adding different doses of clonidine to intrathecal bupivacaine for spinal anesthesia in cesarean section was investigated. It was found that adding 60 µg of clonidine to bupivacaine prolonged postoperative analgesia for longer duration than the addition of 15 and 30 µg clonidine. However, sedation was observed in five patients at five and 15 minutes which reduced to four and three patients at 30 and 60 minutes, respectively, but there was no respiratory depression or fall in saturation. None of the patients from any group required active oxygen supplementation. This result is similar to the findings reported in Bhure et al. and Bhushan et al. [27, 28].

The decrease in the SBP observed in all of BC60, BC30, BC15, and BC10 groups was observed after 45 minutes of infusion of clonidine. On the other hand, a significant dose dependent effect on DBP was observed after 15 minutes of clonidine administration. The decrease in DBP 45 minutes after infusion of clonidine was significantly larger in BC60 group, followed by BC30 and BC15 groups (Figure 2). There was a gradual return to the baseline values within 60 minutes after infusion in all three groups. The BC15 group having lowest dose of clonidine was not statistically different from BC30 (Figure 2). This reflects dose dependent variation in DBP, suggesting increasing dose of clonidine causes greater decrease in DBP. This finding is in accordance with previous studies which indicated no differences in SBP and DBP responses to low doses but responses to higher doses are significantly different [29]. Furthermore, results indicate that there was no statistical significant difference in umbilical artery pH, oxygen partial pressure, and carbon dioxide partial pressure across the groups. In all the three groups, newborns showed no fetal signs of distress which is evident from Apgar scores at one and five minutes, thus confirming the safety adding three different doses of clonidine as adjuvant to bupivacaine.

**Conclusion**

The present study demonstrated that addition of clonidine to bupivacaine significantly improved the onset and duration of sensory and motor block. It is concluded that intrathecal addition of 60 µg of clonidine to bupivacaine prolongs postoperative analgesia for longer duration than 30 or 15 µg of clonidine. However, sedation is more in addition of 60 µg clonidine whereas, less sedation was observed in 30 and 15 clonidine groups, with fairly good quality of analgesia. Hence, the authors suggest that addition of 15 and 30 µg clonidine are better options when sedation is not desirable; on the contrary addition of 60 µg provides excellent quality of spinal analgesia when some amount of sedation is acceptable or required without any deleterious effects on the mother and baby.

**References**


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Pelvic arterial embolization for postpartum hemorrhage: long term results of a single center experience in 29,091 deliveries

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2 Department of Medical Surgical Specialties, Gynaecology and Obstetrics Section, University of Catania, Catania
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Summary

Purpose of investigation: To evaluate the rate of all postpartum hemorrhages (PPHs) treated with uterine embolization in a third level delivery center. Materials and Methods: Since January 2008 to March 2014, 29,091 deliveries were registered in the present hospital in Bergamo, Italy. Among these deliveries, 2,002 cases (6.8%) of PPHs occurred. Seventy-three patients with severe obstetric hemorrhage underwent uterine artery embolization (UAE) (47 cases, 1.61/1,000 deliveries) or hysterectomy (26 cases, 0.89/1,000 deliveries). All identified cases were followed up by telephone on January 2015 in order to evaluate long term results. Results: Embolization was performed successfully in 45 patients (95.7%). Two women underwent total abdominal hysterectomy: one patient for uterine atony and one for adherent placenta. In the follow up all the women interviewed reported the return of their menstrual cycle and 95.2% of women reported regular cycles. Conclusions: Embolization showed a success rate of 95.7%. For this reason, in the authors’ opinion, it is the best choice as second line treatment of PPH, when patient is hemodynamically stable.

Key words: Post-partum hemorrhage; Hysterectomy; Uterine artery embolization; Uterine atony.

Introduction

Postpartum hemorrhage (PPH) is defined as more than 500 cc blood loss after vaginal and more than 1000 cc blood loss after cesarean delivery [1]; it is a common and severe complication of delivery.

PPH causes hypovolemic shock, disseminated intravascular coagulation, renal and liver failure, and acute respiratory distress syndrome [1, 2]. Its main causes are: uterine atony, genital tract lacerations, retained placenta, uterine rupture or inversion, and coagulopathies. [3].

Effective primary management requires the use of uterotonic agents, suturing lacerations, fundal massage, uterine cavity revision, bimanual uterine compression, and uterine tamponade. In case of failure of these procedures, ligation of hypogastric vessels, compressive suture (as B-Lynch uterine compression) or hysterectomy are generally performed [3].

In selected cases, embolization is an alternative to hysterectomy. Uterus and fertility preservation represent the main advantages of this procedure [4]. Uterine embolization became a common procedure in the last decades; however, few large series have been published on its middle- to long-term outcomes.

In this manuscript, the authors present the results of all PPHs treated with uterine embolization in a third level delivery center in Bergamo, Italy.

Materials and Methods

This is a retrospective analysis of patients who delivered and underwent uterine artery embolization (UAE) due to PPH in the period between 2008 and 2014 at the “Papa Giovanni XXIII” Hospital, in Bergamo, a third level delivery center in Italy. During the period from January 2008 to March 2014, 29,091 deliveries were registered in the hospital. Among these deliveries, 2,002 cases (6.8%) of postpartum hemorrhage (> 500cc) occurred (Table 1).

According to the hospital protocol and national guidelines, 73 patients with severe obstetric hemorrhage, resistant to medical treatment and manual or instrumental uterine revision (in case of vaginal birth), underwent (47 cases, 1.61/1,000 deliveries) or hysterectomy (26 cases, 0.89/1,000 deliveries). Women received UAE only if they were hemodynamically stable [5-8]. In other cases progressive vessel ligation and compressive suture were performed; when these attempts were not successful, hysterectomy

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was the chosen treatment. This study reports only cases of uterine embolization. All uterine embolizations were carried out in the operative room of the Radiology Department of the “Papa Giovanni XXIII” Hospital of Bergamo. For all cases the following data were retrieved by evaluation of clinical records: maternal age, gestational age at birth, parity, mode of delivery, epidural analgesia, episiotomy, birth weight, hemoglobin value, number of packed red blood cell or platelet transfusions, failures of embolization, and complications. The cause of hemorrhage was evaluated: uterine atony, abruptio placentae, placenta praevia, cervix injury, placental accreta, and percreta, myoma (diagnoses accepted by obstetricians and sometimes confirmed by a histological exam).

All identified cases were followed up by telephone in January 2015 in order to evaluate long term results. The patients were asked about any complication after embolization procedure, return of normal menstrual cycle, and presence of pelvic pain. Furthermore they were asked: ‘Did you become pregnant after the procedure?’, ‘If yes, when and how did you give birth?’; ‘If not, do you intend to have more children?’ Follow up data were obtained from 42 subjects. In five cases the patients were lost at follow up.

**Embolization procedure**

UAE for the treatment of PPH was performed only after all usual obstetric maneuvers for the treatment of PPH and in hemodynamically stable patients. The patients were transported to the Radiology Department (ten minutes from the delivery room), accompanied by a gynecologist and an anesthesiologist. An interventional radiologist performed each procedure. The femoral artery was cannulated and an angiogram provided a roadmap for the catheter as it was maneuvered into uterine arteries. The embolic agent was released into both the right and left uterine arteries by repositioning the same catheter that was originally inserted. At the end of the procedure, the catheter was removed and pressure was applied to stop any bleeding. The area of the skin where the catheter was inserted was then covered with a dressing.

---

**Table 1. — Characteristics of patients, deliveries, and causes of hemorrhage.**

<table>
<thead>
<tr>
<th></th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>30 (range: 22 – 39)</td>
</tr>
<tr>
<td>Multiple pregnancies</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>8 (17%)</td>
</tr>
<tr>
<td><strong>Mode of delivery</strong></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>24 (51%)</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>23 (49)</td>
</tr>
<tr>
<td>Elective cesarean section</td>
<td>11 (23%)</td>
</tr>
<tr>
<td>Urgent cesarean section</td>
<td>12 (25%)</td>
</tr>
<tr>
<td>Epidural anesthesia</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Perineal laceration grade I-II</td>
<td>8 (17%)</td>
</tr>
<tr>
<td><strong>Causes of hemorrhage</strong></td>
<td></td>
</tr>
<tr>
<td>Uterine atony</td>
<td>28 (60%)</td>
</tr>
<tr>
<td>Retention of placenta</td>
<td>9 (19%)</td>
</tr>
<tr>
<td>Adherent placenta</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Incomplete placental expulsion</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Amniotic acute infection</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Intrauterine fetal death</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Figure 1. — Algorithm of deliveries and PPH.

**Results**

The characteristics of the 47 cases are shown in Table 1. Forty-four women underwent the procedure following primary PPH. In three cases, bleeding occurred at respectively, 36 hours, 60 hours, and 13 days after delivery. One patient had both intrauterine fetal death and amniotic acute infection as cause of PPH.

The mean value of hemoglobin, measured before the procedure was 8.9 g/dl (range 4.5 to 14.4). All patients underwent blood transfusion before the procedure: mean four bags of packed red blood cells (range one to 14) and two fresh frozen plasma (range one to ten).

**Procedures before embolization**

The procedures and/or drugs to resolve the hemorrhage before embolization were: syntocinon (10–50 IU) in 23 cases (48.9%), naldor (1–3 fl) in 33 cases (70.2%), cytotec (800 mg) in two cases (4.2%), calcium gluconate (2 fl) in six cases (12.7%), and tranex (2 fl) in one case (2.1%).

Uterine tamponade was performed in 26 cases (55.3%),
Table 2. — Long term follow-up.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic pain</td>
<td>1/42 (2%)</td>
</tr>
<tr>
<td>Lower limb circulatory disorder</td>
<td>1/42 (2%)</td>
</tr>
<tr>
<td>Return of the menstrual cycle</td>
<td>42/42 (100%)</td>
</tr>
<tr>
<td>Regular menstrual cycles</td>
<td>40/42 (95%)</td>
</tr>
<tr>
<td>Plan to become pregnant again</td>
<td>21/42 (50%)</td>
</tr>
<tr>
<td>Pregnancy after embolization</td>
<td>3/42 (7%)</td>
</tr>
</tbody>
</table>

Long term results

All treated women reported return of menstrual cycle (42/42; 100%) after the procedure and almost all in a regular way (40/42; 95.2%). This rate is consistent or higher than previously reported [8, 9]. Out of the three women who had a subsequent pregnancy, only two had a term delivery: in one case PPH also occurred in the second birth, requiring embolization and then hysterectomy; in the other case, the cause of bleeding was a placenta accreta with subsequent hysterectomy.

Discussion

The aim of this study was to evaluate the effectiveness of uterine embolization in the treatment of uterine PPH and the middle- to long-term results of it.

In order to identify a homogeneous group of patients, the authors included only women who delivered at their hospital, not considering patients treated in their center, but delivered in other hospitals. The management of PPH could differ in different centers. In the authors’ delivery room, in accordance with Italian and international guidelines (ACOG, RCOG), uterine embolization was considered only in case of failure of first line medical treatment and procedures, and after ensuring the patient’s hemodynamic stability.

The rate of PPH observed in this study is similar to the rate reported in the literature [10]. Likewise the rate of post-partum hysterectomy observed in the authors’ centre in the considered period is largely similar to the rates reported in the same period in other Italian hospitals [5]. In the present data, 60% of PPH were caused by uterine atony. This figure is consistent with the data reported in literature, showing percentages ranging from 45% to 78% [4, 6, 7, 10]. The success rate of embolization was 95.7%. Studies conducted in different countries have reported success rates ranging between 73% and 100% [5, 10-15].

In the present series, embolization failure was observed in one case of uterine atony and in one case of adherent placenta. In a study conducted between 1996 and 2001 by Tourné et al. [7], among 12 embolization procedures, the only hysterectomy was performed in a case of retained placenta.

In the present study, no patients required a second embolization. In the literature a second embolization has been reported ranging from 8% to 15% of cases [5, 11, 13, 14]. The major complications of embolization procedure reported in the literature are: dissection, allergy to contrast, hematoma at the injection site of the catheter and pain [11,13,16]. In this study none of these complications occurred.

Conclusions

In conclusion, in this study embolization showed a success rate of 95.7%. For this reason, in the authors’ opinion, it is the best choice as second line treatment of PPH, when patient is hemodynamically stable.

The results at distance of the present cases showed that in all cases, regular menstrual cycles were restored: all
women have maintained their reproductive potential and in two cases the patients obtained a second birth. This underlines the role of embolization procedure in preserving fertility.

References


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Introduction

Menopause is a natural process that occurs in women as a normal part of aging [1]. The average age of menopause is 51 years. Menopause causes dramatic hormonal changes, such as the ablation of estrogen, which can affect the immune-regulatory system. Estrogens, which are female sex hormones, regulate growth, differentiation, and the function of many reproductive tissues. During the menopausal period, estrogen levels are chronically reduced. As a result, impairment of immune functions [2], osteoporosis [3], cardiovascular diseases [4], cognition, learning and memorization [5], and many neurodegenerative problems can occur [6].

Bortezomib (BORT) is a novel proteasome inhibitor that has been used in preclinical and phase 1 studies and has been shown to induce anti-myeloma activity. Normally, proteasome activity is important for healthy cell life and cycles. It is known that proteasome activity is effective in the regulation of some protein groups which play an important role in cell cycle control, the beginning of the transcription process, apoptosis, and intracellular signalling, as well as not-required (incorrectly synthesized, damaged, or oxidized) proteins [7]. Actually, proteasome inhibition leads to the suppression of nuclear factor kappa B (NF-κB), which is one of the most important intracellular pathways [8]. On the other hand, the suppression of NF-κB can inhibit regulation of the transcription of various genes which code the pro-apoptotic and anti-apoptotic proteins of apoptosis, cell proliferation, growth factors, cytokines, and cell adhesion molecules [9].

Proteasomal pathway inhibition may lead to a variety of effects at the cellular and tissue levels, such as aging, geriatric diseases, BORT resistance, deficiency of estrogen, and some transcription factors. In the current literature, there are few studies that include the effects of BORT on uterine tissue. One of these studies demonstrated that BORT treatment had positive effects on a patient who was suffering from uterine cancer [10]. In addition, the authors showed that it led to an increase in cell death in the endometrial carcinoma cell line under in-vitro conditions [11]. The present study is the first experimental study that aimed to show the possible effects of BORT treatment on uterine tissue. The possible effects of BORT treatment on uterine tissue were investigated by using morphometric, histopathological, and immunohistochemical methods.
**Materials and Methods**

**Animals and experimental groups**

The animals used in this study were kept in facilities accredited by international guidelines and the studies were approved and conducted in accordance with the Institutional Animal Care and Use Committee of Atatürk University. Eighteen adult (12-week-old) female Sprague-Dawley rats were used from the Atatürk University Experimental Animal Laboratory (ATADEM). The animals were housed in groups of six per cage for at least seven days under controlled conditions of constant temperature/humidity and were exposed to a 12-hour light/dark cycle. The rats were randomly allocated into three groups: 1) healthy control group (group 1, n = 6), 2) ovariectomized (OVX) group (group 2, n = 6), and 3) OVX + BORT group (group 3, n = 6).

**Experimental models**

**OVX procedure**

A bilateral OVX was performed by making a longitudinal incision (0.5–1 cm) in the midline area of the lower abdomen, removing the ovaries, and closing the skin incision. For the two days following OVX, 25 mg/kg of metamizol sodium was administered to the rats as an analgesic. The OVX rats were separated into two groups (group 2 and 3). The rats in Group 2 were kept alive for 12 weeks. On the eighth week, 0.2 mg/kg of BORT was intraperitoneally applied to group 3, twice a week for a month [12].

**Research methods**

**Histological examination**

Each uterus was fixed in 10% formalin solution for 48–55 hours, dehydrated in a graded alcohol series, embedded in paraffin wax, and sectioned using a microtome. For light microscopic histological examination, sections were stained with Hematoxylin & Eosin (H&E). The slides were covered and photographs were taken using a light microscope with a camera attachment.

H&E staining was performed on ten sections with five-μm thicknesses that were obtained systematic randomly from each group’s specimens (ten sections were examined in the sampling range of 1/10. After the first section, 11 sections were used. Therefore, tissue samples were obtained in the range of 500 μm). Endometrium and myometrium thicknesses were computed under a ×4 objective microscope.

**Immunohistochemical staining of NF-κB**

Paraffin-embedded lung samples were used for a p65 subunit of NF-κB immunohistochemistry. Tissue sections of five-μm were deparaffinised in xylene and rehydrated in ethanol followed by water and phosphate-buffered saline. Endogenous peroxidase was blocked by immersion in 3% hydrogen peroxide. The tissue sections were then incubated with NF-[kappa] B antibody at a concentration of five µg/ml for one hour at room temperature. Control sections were incubated with phosphate-buffered saline containing normal goat serum without a primary antibody. Immunostaining was then detected with a streptavidin-biotin complex kit and developed with diaminobenzidine tetra-hydrochloride. The sections were counterstained with Mayer hematoxylin followed by light microscopy.

**Statistical analysis**

Data were analyzed using the Statistical Package for the Social Sciences (SPSS version 19.0) Software. A comparison of wall thicknesses of the endometrium and myometrium was performed using ANOVA. A p < 0.05 was considered significant.

**Results**

**Morphometric results**

The results regarding endometrial and myometrial wall thickness are given in Table 1 and Figure 1. When endometrial thicknesses were evaluated, there were significant differences between the OVX and OVX + BORT groups when compared with the control group. There were also significant differences between the OVX and OVX + BORT groups (p < 0.05).
The protective effect of the proteasome inhibitor bortezomib on the uterus of ovariectomized rats

Regarding myometrial thickness, there were significant differences between the OVX and OVX + BORT groups when compared with the control group (p < 0.05), but there were no significant differences between the OVX and OVX + BORT groups (p > 0.05).

Histopathological results

Control group
All layers of the uterine histological structure had a normal appearance. That is, the endometrium, myometrium, and perimetrium were distinctly observed. The lumen of the uterus was centrally located (Figure 1A). The lamina epithelium of the endometrium was composed of simple columnar epithelium and its euchromatic nucleus had a normal appearance (Figures 2A-B). The vascular structure and glandule uterine, which were located on the endometrium, appeared normal (Figure 2A). The circular and longitudinal smooth muscles of the myometrium layer had a normal appearance (Figure 1A).

OVX group
When histopathological changes were determined in the OVX group, there were some marked differences in the endometrium (Figure 1B) and myometrium. For example, the epithelial layer of the endometrium had changed into simple cuboidal or squamous epithelial cells (Figure 2D). Inflammatory cell infiltration was observed in the endometrium (Figure 2C). There was a decrease in the thickness of the myometrium as compared to the control group. The muscle cells of the myometrium had small and hyperchromatic nuclei.

OVX + BORT group
In this group’s sections, general uterine structure was protected when compared with the OVX group. The epithelial layer of the endometrium was composed of simple columnar cells; however, in some areas, there were limited cuboidal and squamous cells (Figure 2F). There was a decrease in the density of the inflammatory cells’ infiltration of the endometrium as compared to the OVX group (Figures 2E-F). The smooth muscle cells of the myometrium had small and hyperchromatic nuclei.

Immunohistochemical results

In the control group’s section, NF-κB p65 immuno-positivity was only observed in the vascular structure and endothelial cells (Figures 3A-B). In the OVX group, many of the endothelial cells were immune-positive. Endometrial stromal cells had also a slight immuno-positive reaction for NF-κB p65 (Figures 3C-D). When looking at the OVX + BORT group’s section, there were a few immuno-positive cells (Figures 3E-F).

Discussion

In this study, the authors examined the effects of administering BORT, a proteasome inhibitor agent, to the uterus in the post-menopausal period using morphometric, histopathological, and immunohistochemical methods. In the first part
of this evaluation, a significant increase was detected in the level of uterine weight against estrogenic activity when the various estrogen treatments' effects on OVX rats were comparatively examined. This case was expressed as an increase in the thicknesses of the endometrium and myometrium. The myometrium had more improved than normal under the estrogen effects. This improvement was proven by both the growth and proliferation of muscle cells [13]. Many smooth muscle cells began to have the ultrastructural features of protein-secreting cells related to their estrogen usage and actively synthesized collagen. Thus, the uterine collagen level significantly increased [14-16].

There was a significant decrease in endometrial thickness in the experimental groups when compared with the control group. The highest level of endometrial thickness was 526.16±126.64 µm, which belonged to the control group. The lowest level of endometrial thickness was 137.41±62.29 µm, which belonged to the OVX group.

In this case, it has been shown that OVX and the disappearance of female sex hormones had a significant impact on the structural elements of a single epithelial layer and the connective tissue of the endometrium. This effect is probably emerging as a quantitative decrease in blood vessels, collagen amounts, and connective tissue cells. It was detected that endometrial thickness of the OVX + BORT treatment group (236.67±35.95 µm) was statistically different from the other two groups, but it was less than 526.16±126.64 µm in the control group and more than 137.41±62.29 µm in the OVX group. In other words, it was observed that BORT treatment inhibited the negative effects of OVX on endometrial thickness.

The second part of the present discussion focused on histological detections. In their histopathological study, Fawcett et al. [17] observed 1) atrophy on OVX and non-treated rat uteri, 2) the transformation of prismatic epithelium to cubic epithelium, 3) atrophy in endometrial glands, 4) regulation of stromal connective tissue fibres to tight-intensive form, and 5) shrinkage and hyperchromasia of stromal cells and smooth muscle cells [18, 19].

In a study by Iguchi et al., the authors proved that atrophic changes occurred in uterine smooth muscle [20, 21]. Regarding this issue, the present study is consistent with the current literature data. In addition, BORT treatment may be morphometrically and histopathologically protective. The natural structure of the uterus was protected by itself when the OVX + BORT treatment group was histologically examined.

The third part of the present discussion focused on the immunohistochemical results. Normally, IκB is phosphor-ylated on the terminal serine residues and thus is targeted to poly-ubiquinone. Then it is degraded by the proteosomal pathway. The degradation of IκB enables NF-κB to enter into the core as a transcriptional factor and bind to the target gene region on the DNA [22, 23]. However, there is a second pathway in which NF-κB can be activated. In this case, there is no need for proteasomal degradation of IκB. Therefore, it is not affected by a proteosome inhibitor such as BORT. This is called an atypical pathway. It is not necessary for NF-κB to enter into the core by nuclear transcription and be activated by proteosome in an atypical pathway. This second NF-κB pathway depends on phospho-
tyrosine. Aging may be responsible for chronic activation of NF-κB. Therefore, NF-κB induction is thought to be one of the cellular effects of aging that may lead to activation of the atypical pathway [24].

In the present study, immunopositivity was not observed on the muscle bundles of muscular layers, uterine glands, and single epithelial layer of the endometrium of the uterus in all experimental groups, when NF-κB p65 immunoreactivity was evaluated on the endometrium, myometrium, and perimetrium layers of the uterus. One of the common findings in all the present study groups was that NF-κB p65 immunoreactivity was clearly positive only in blood vessels and endothelial cells. Strong nuclear and cytoplasmic NF-κB involvement was observed in the stromal cells of the endometrium, especially in some stromal cells in the endometrium basalis in the O VX group. NF-κB immunoreactivity was evident in the stromal cells but cytoplasmic involvement was related to the OVX + BORT treatment group.

In light of the data the present authors obtained, they can say that first there was marked degeneration in the endometrium (connective tissue) and myometrium (smooth muscle tissue) layers of the uterus during the menopausal period, as well as a lack of sex hormones (especially estrogen). In other words, the preventive and curative effects of BORT (proteasome inhibition) may be partially considered. The current literature indicates that proteasome inhibition (BORT treatment) is equal to NF-κB inhibition [11]. As a consequence, this study demonstrated that OVX will have some adverse effects on the uterus (as in the literature) [18-19]. A limited preventive effect of proteasome inhibition was detected by using morphometric and immunohistochemical methods. This study can contribute two important findings to the literature on BORT: first, estrogen deficiency activates the NF-κB pathway in the endometrium, and second, NF-κB exhibits a cytoplasmic reaction in a proteasome-independent way.

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Clinical efficacy of fiberoptic ductoscopy in combination with ultrasound-guided minimally invasive surgery in treatment of plasma cell mastitis

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Summary
Objectives: To analyze clinical efficacy between fiberoptic ductoscopy plus ultrasound-guided minimally invasive irrigation and lesion resection in treating plasma cell mastitis (PCM), aiming to provide clinical evidence for treating PCM. Materials and Methods: 119 patients undergoing fiberoptic ductoscopy plus ultrasound-guided minimally invasive surgery in Ningxia People’s Hospital were allocated into the breast duct irrigation group, and 95 counterparts receiving lesion resection in the Affiliated Hospital of Ningxia Medical University into the control group. Clinico-pathological characteristics and therapeutic effect were compared between two groups. Results: The cure rate in the breast duct irrigation group was 98.31% (117/119), significantly higher than 90.53% in the control group (p < 0.05). In the breast duct irrigation group, overall treatment time was 20.13 days, significantly longer than 15.15 days in the control group (p < 0.05). During postoperative follow-up, no recurrence was observed, significantly lower compared with 48.8% (42/86) in the control group. The degree of satisfaction in the breast duct irrigation group was 95.79% (114/119), significantly higher compared with 74.74% (71/95) in the control group. Conclusions: Fiberoptic ductoscopy plus with ultrasound-guided minimally invasive drainage is a novel and effective treatment of PCM with high cure rate, low recurrence rate, slight pain, and effectively maintains breast integrity.

Key words: Plasma cell mastitis; Surgical operation; Fiberoptic ductoscopy; Minimally invasive drainage; Ultrasonics.

Introduction
Plasma cell mastitis (PCM) is a chronic non-bacterial breast inflammatory disease and mainly occurs during non-puerperal period, which is characterized as duct ectasia and plasma cell infiltration [1]. PCM is probably caused by acute sterile inflammatory response induced by accumulation of lipid secretion in the breast duct [2]. The pathogenesis of PCM is complicated and clinically characterized as breast tumor accompanied with/without topical redness and swelling, inflammatory lesions, mammary areola abscess, mammary duct fistula, etc. The degree of inflammation is inconsistent with clinical symptoms with slight or no systemic symptoms. Along with the progression of pathological changes, the presence of inflammatory responses, dominantly plasma cell infiltration, is defined as PCM. It is likely to make a misdiagnosis of PCM. Traditional surgery yields poor clinical efficacy and high recurrence rate, and is likely to destroy the breast morphology. Albeit combined therapy of fiberoptic ductoscopy and traditional Chinese medicine has been reported recently, the clinical efficacy remains elusive. A total of 119 patients pathologically diagnosed by PCM cell smears or core needle biopsy in Department of Breast Surgery, Ningxia People’s Hospital between June 2011 and June 2013 were enrolled in this study. All participants underwent fiberoptic ductoscopy irrigation and minimally invasive drainage (abbreviated as breast duct irrigation group). Another 95 PCM cases were surgically treated with lesion resection in the Affiliated Hospital of Ningxia Medical University (control group). The therapeutic effect was retrospectively analyzed and statistically compared between two groups, is reported.

Materials and Methods
General data
Clinical data of 119 PCM patients in the breast duct irrigation group were collected including two married males, 117 female (three single, three pregnant, eight married but nulliparous, and 105 lactation), aged 12-47 years, with a median age of 36 years. Among them, 49 (41.18%) were affected on the left side, 63 (52.94%) on the right side, and seven (6%) with bilateral involvement; 112 cases (94.12%) had single lesion and seven (6%) presented with multiple lesions. Yellow or gray turbid discharge was observed in 87 patients (73.11%) and no discharge found in the remaining 32 cases (26.90%). The course of diseases ranged from 11 d to four months, 32 days on average. Eighty patients (67.23%) had inverted nipple and 27 presented with breast masses (mammary areola masses in 20 and peripheral masses in seven) and 43 had pus; 12 patients presented with single fistulization and five had fistulization in over two sites. Seventy-nine patients (66.39%) had surgical history of tumor excision. Two cases were subject to inverted nipple correction, 23 (19.33%) were taking traditional Chinese medicines for over three months, and one received acupuncture for more than two months.
Other 95 PCM cases surgically treated with lesion resection in the Affiliated Hospital of Ningxia Medical University were assigned into the control group, 93 female and two males, 37 years on average. The gender, age, obstetrical history, type of pathological changes, and course of diseases were matched between two groups (p > 0.05). The time of follow-up ranged from six to 36 months in both groups. This research protocol was approved by the Ethics Committee of Ningxia Medical University before the study was initiated. Written informed consents were obtained from all participants in this study.

Treatment methods

Breast duct surgery: The diagnostic criteria were determined according to the staging of disease development [3]: stage I effusion accumulated in the breast duct; stage II masses formed; stage III pus accumulated in the lesions; stage IV formation of skin ulceration sinus tract. Different therapies targeting each stage were performed and rehabilitation treatment was delivered subsequently. (1) Thirty-two cases with mammary duct ectasia effusion were treated with fiberoptic ductoscopy irrigation alone. (2) For patients with gray or yellow discharge in the nipple, B-ultrasound revealed signs of punctate strong echo in the breast duct ectasia of the masses. Slight migration was sensed under probe compression. Twenty-seven cases had inactive primary inflammatory tumors characterized with no envelope, unclear margin and gland edema, and underwent fiberoptic ductoscopy irrigation under direct vision. (3) Forty-three patients with breast abscess were subject to fiberoptic ductoscopy irrigation in combination with minimally invasive drainage irrigation. Approximately two to ten syringes were implanted guided by B-ultrasound based on single or multiple abscesses, making the injection and outflow in synchronization. The depth and position of the syringes were adjusted based on B-ultrasound monitoring and the resistance of injection and outflow to guarantee thorough drainage of abscesses, accelerate wound healing, and avoid the formation of sinus tract. No formation of sinus tract occurred in 43 patients with breast abscesses throughout the whole treatment. (4) Seventeen cases with breast duct fistula were treated with fiberoptic ductoscopy irrigation in combination with minimally invasive drainage irrigation. The normal skin surrounding the ostia was chosen as the site of needle implantation rather than sinus tract opening for patients with formation of sinus tract. The ostia was selected as the opening of outflow. After irrigation, moist burn ointment (MEBO) was daubed and sterile honey were daubed on the ostia to accelerate ostia healing. The depth and thickness of retained syringe were adjusted based upon B-ultrasound findings, depth, and diameter of the sinus tract. Seventeen patients presenting with sinus tract formation were all completely healed, whereas the course of treatment was significantly longer compared with their counterparts in abscess and tumor stage.

Fiberoptic ductoscopy irrigation: ornidazole or 0.9% sodium chloride solution was injected through the discharge hole to dilute the substance accumulated in the breast duct. For those with crystal formation, melting chymotrypsin was injected for 15-minute perfusion and then discharged. The frequency of irrigation was determined based upon the property and quantity of the substance accumulated in the breast duct. Patients with a slight amount of accumulated substance received single irrigation. Those with a large amount of sticky and even crystal substance were subjected to two to three times irrigation. The syringe was kept for irrigation: the position of the catheter was be validated according to B-ultrasound findings before irrigation.

Irrigation sequence: the syringe needle was inserted into the needle plug and the discharge was removed using 0.9% sodium chloride solution to avert the incidence of retrograde flow, and then the syringe was inserted into the needle plug and 0.9% sodium chloride solution, 0.5% ornidazole and 80,000 units of gentamicin were injected into the lesions in sequence. During the process of irrigation, the condition of cavity was monitored under B-ultrasound, the injection resistance was sensed by hands, and the degree of outflow was observed by vision. Whether the syringe was retained depended upon the size of cavity, the brightness, and quantity of effusion.

During the entire course of treatment, partial patients presenting with fever and worsened inflammation during the early stage of PCM were administered with penicillin, combined with ornidazole via intravenous injection. Immunity enhancers, such as thymosin, traditional Chinese medicine which removes the heat and toxicity and systemic treatment, were delivered during topical perfusion and irrigation.

Lesion excision: Sixteen patients underwent breast lobe resection, 29 topical wedge resection, 31 excision and drainage of abscess, five simple mastectomy, and 14 resection of chronic sinus tract and fistula.

Statistical analysis

SPSS 13.0 software package was utilized for statistical analysis in this study. Measurement data were expressed as mean ± standard deviation. The overall time of treatment was statistically compared between two groups by independent sample t-test and enumeration data were statistically compared by chi-square test. The ordinal data were analyzed by rank-sum test. A p < 0.05 was considered as a statistical significance.

Results

Total time of treatment

The total time of treatment differed in 119 PCM patients in the breast duct irrigation group: 8, 12, 26, and 41 days for patients in mammary duct ectasia effusion, breast tumor, breast abscess, and breast duct fistula stages, respectively. The mean total time of treatment was 20.13 days, significantly longer compared with 15.15 days in the control group (p < 0.05), as illustrated in Table 1.

Therapeutic effect and follow-up

Following breast duct irrigation, 117 patients recovered and two underwent total mastectomy with a cure rate up to 98.3%, significantly higher than 90.53% (86/95) in the control group. Referring to the treatment criteria widely accepted by Chinese physicians [4], the patients eligible for the healing criteria were re-examined by ultrasound test at one week, one, three, six, and 12 months until the registering date (at least one year). In the breast duct irrigation group, merely two cases showed slight topical discomforts, which were alleviated by B-ultrasound-guided suction of turbid residue and repeated irrigation with ornidazole so-
Clinical efficacy of fiberoptic ductoscopy in combination with ultrasound-guided minimally invasive surgery in treatment of plasma cell mastitis

The recurrence rate in the breast duct irrigation group was significantly lower compared with 48.4% in the control group (Table 2).

**Postoperative changes in breast shape**

According to the criteria evaluation criteria [5], excellent: the treated breast almost resembled the contralateral side in size and shape; good: breast retraction and/or skin alterations involving with < 1/4 of the original breast; average: breast retraction and/or skin alterations involving with 1/4 to 1/2; poor: breast deformity involving with > 1/2. The breast shape in the breast duct irrigation group was significantly superior to that in the control group ($p < 0.05$), as illustrated in Table 3 and in Figures 1-4.

**Degree of satisfaction of patients**

Self-designed questionnaire was adopted to evaluate the

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**Table 2.** Comparison of clinical efficacy and recurrence rate between two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment efficacy (cure rate)</th>
<th>Not healed</th>
<th>Recurrence rate (recurrence rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast duct irrigation group</td>
<td>117 (98.31%)</td>
<td>2</td>
<td>117</td>
</tr>
<tr>
<td>Control group</td>
<td>86 (90.53%)</td>
<td>9</td>
<td>44</td>
</tr>
</tbody>
</table>

Note: Albeit this therapy has been rarely conducted in China, none of the 117 PCM patients undergoing fiberoptic ductoscopy in combination with minimally invasive drainage recurred during the follow-up with a recurrence rate of 0.

**Table 3.** Evaluation of postoperative breast deformation between two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases (n)</th>
<th>Changes in breast shape [n(%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast duct irrigation group</td>
<td>119</td>
<td>Excellent 34</td>
</tr>
<tr>
<td>Control group</td>
<td>95</td>
<td>Excellent 7</td>
</tr>
</tbody>
</table>

Note: $Z$-value 4.586, $p$-value 0.000

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**Figure 1.** — PCM patient before breast duct irrigation.

**Figure 2.** — PCM patient after breast duct irrigation.

**Figure 3.** — PCM patient before surgical excision treatment.

**Figure 4.** — PCM patients after operation.
patients' degree of satisfaction. The degree of satisfaction for therapeutic effect, service attitude, and breast shape achieved to 95.8% in the breast duct irrigation group, significantly higher than 74.74% in the control group ($p < 0.05$), as shown in Table 4.

### Discussion

Based on the aforementioned results, the cycle of breast duct irrigation was 20.13 days, significantly longer than 15.15 days of lesion excision because different therapies were selected according to the staging and development of diseases in the breast duct irrigation group. In this study, nipple dysplasia, epithelial hyperkeratosis or breast duct occlusion and ischesis induced by galactostasis, duct ectasia, galactophoritis, chronic granuloma mastitis, and even secondary abscess and fistula were the potential causes and pathogenesis of the development of pathological changes of varying degree [6]. The direct vision and infusion of fiberoptic ductoscopy were fully utilized to directly and repeatedly inject chymotrypsin and ornidazole or 0.9% sodium chloride solution into the cavity and to dissolve and eliminate the ischesis within the necrotic cavity, which avoided the biochemical reaction aroused by the effusion accumulated in the breast duct and prevented the progression to tumors even the formation of pyogenic sinus tract [7]. The repairing process of the lesions and the integrity of breast healing were fully considered. The present authors suggested that it is more likely to be accepted by the patients as compared with breast tissue defected caused by surgical excision.

In terms of cure rate, recurrence rate and patients' degree of satisfaction, fiberoptic ductoscopy in combination with ultrasound-guided minimally invasive drainage is a rational and reliable therapy. Repeated dissolution and irrigation of the lesions combined with B-ultrasound guided implantation of venous needle can yield accurate and direct drainage and significant advantages in drainage. Establishment of a channel with an injection entry from fiberoptic ductoscopy and an exit from drainage tube is able to maintain the short-term cure rate as high as 98.31%. After healing, the patients should be closely monitored by B-ultrasound during subsequent follow-up. The effusion accumulated in the breast duct could be fully removed under fiberoptic ductoscopcy. The topical residual or new effusion with the diseased cavity surrounding the breast duct could be timely extracted to minimize the recurrence of diseases. However, the lesion may be directly resected by surgical approaches, but the effusion outside the breast duct and the potential lesions in alternative sites could not be fully eliminated, which severely affects the healing of PCM and leads to a high recurrence rate up to 48.84% in the control group of current study. In addition, repeated surgeries cause serious breast tissue defects and distortion.

Compared with conventional surgery, irrigation drainage is a historic revolutionary treatment of PCM from traditional lesion incision and drainage or surgical tissue excision to minimally invasive drainage and tissue healing under fiberoptic ductoscopy with an incision of one to two mm, which significantly alleviates skin injury, tissue defects, and slight scars after healing. More importantly, this technique maintains the integrity if the breast as much as possible. In addition, it causes significantly less pain both physically and mentally compared with surgical incision, drainage, and excision. All these advantages contribute to the high patients' degree of satisfaction, up to 95.80% obtained in this group. Alternative causes possibly included: first, the healing effect was recognized by most patients; second, minimally invasive was widely accepted in modern medicine; third, concentrated therapy offered confidence to patients in persisting the treatment by mutual visit, exchange, comforting, and encouragement. The major limitation is that a slight proportion of patients may give up the therapy due to the relatively long operating time, which remains to be improved by intensive communication with nationwide PCM specialists to obtain valuable clinical experience. The more advanced five-channel tube should be applied in fiberoptic ductoscopy irrigation, which integrates biopsy, vacuum suction of effusion, excision of necrotic tissue, and implantation of drainage tube in one-step procedure and significantly shortens the course of treatment of PCM.

Taken together, previous studies found that the pathogenesis of PCM is the effusion in breast duct, immune hypofunction, anaerobe infection, etc. In this study, the PCM patients underwent fiberoptic ductoscopy irrigation in combination with ultrasound-guided minimally invasive drainage and immunity-enhancing therapy, which corresponds to the underlying pathogenesis. It is equally advantageous in terms of overall clinical efficacy and the integrity of breast morphology, etc. Previous studies demonstrated that surgery is the most radical and effective treatment of PCM [8], whereas it is likely to destroy the breast morphology. In this study, the recurrence rate of PCM in the control group was up to 48.84%, leading to severe psychological pain during daily life. Moreover,

### Table 4. Comparison of degree of satisfaction of PCM Patients between two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases (n)</th>
<th>Satisfied</th>
<th>Not satisfied</th>
<th>Degree of satisfaction (%)</th>
<th>$\chi^2$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast duct irrigation group</td>
<td>119</td>
<td>114</td>
<td>5</td>
<td>95.80%</td>
<td>20.003</td>
<td>0.000</td>
</tr>
<tr>
<td>Control group</td>
<td>95</td>
<td>71</td>
<td>24</td>
<td>74.74%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the average age of onset in patients was 36 years. Maintaining the breast integrity not only contributes to building a stable and harmonious family, but also enhances the survival and quality of life of patients. Chinese medicine treatment has also been recommended by certain scholars [9]. However, 19.33% (23/119) of patients in this study were treated with Chinese medicine for six months to two years. One case receiving acupuncture therapy for half a year presented with pyogenic necrosis evolving from redness and swelling. Hence, the clinical efficacy of traditional Chinese medicine in treating PCM remains to be further elucidated. In the current investigation, ultrasonic intervention and guidance played a significant adjuvant role in treating PCM. In particular, compared with traditional "cross" incision and drainage by placement of vaseline gauze, the ultrasound-guided implantation of needle yielded a smaller wound for drainage, more accurate position, adjusted the position and depth of the needle in a timely manner, guaranteed the success of drainage, facilitated subsequent irrigation, and reduced the pain during dressing changes. Intracavity irrigation once daily is able to discharge the ischesis within the necrotic cavity more directly and quickly compared with conventional surface dressing change two to three times per day. Non-traumatic physiotherapy, hot compress, and vacuum suction should be supplemented to consolidate the therapeutic effect. B-ultrasound-guided monitoring, suction, and irrigation play a pivotal role in preventing the recurrence of PCM. The clinical efficacy of this technique has been widely recognized by the clinicians and patients from many provinces and autonomous regions, such as Ningxia, Shanxi, Gansu and Inner Mongolia, etc.

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Couple-related factors of ART outcome

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Introduction

Assisted reproductive technologies (ART) have been successfully used to treat infertility for several decades, where mainly in vitro fertilization (IVF) and its submethod intracytoplasmic sperm injection (ICSI) [1] have yielded clinical pregnancies in up to 30% of treated patients [2].

The outcome of both IVF and ICSI depends on several factors such as age, BMI, lifestyle factors, and concomitant diseases in one or both partners [3, 4], but also low response to hormonal pre-IVF stimulation, poor embryo quality, as well as early post-IVF miscarriage [5]. Inflammatory diseases of the urogenital tract have also been shown to increase the risk of infertility, miscarriages or preterm labor [6, 7], with bacterial vaginosis being one of the most prevalent diagnoses in women [8, 9] and (chronic) prostatitis in men [10, 11]. However, the effect of either condition on IVF/ICSI outcome is uncertain and available results non-conclusive [12-15]. Also, there are very few studies where both counterparts of the couple have been simultaneously studied, although such data may turn out to be more informative and potentially leading to higher public awareness and better options in determining avoidable or treatable causes of infertility.

Therefore, the aim of this study was to determine possible health-related risk factors in both counterparts of the couple affecting the IVF/ICSI outcome, including the possible effect of bacterial vaginosis and/or prostatitis.

Materials and Methods

Study group

The study group consisted of couples undergoing IVF or ICSI procedure in one fertility clinic in 2011, recruiting 100 consecutive couples who were willing to participate and thereafter gave an informed consent for study-related procedures. Study subjects’ age ranged from 25 to 58 years (average 35.7), with female partners being significantly younger than their male counterparts (34.1 vs. 37.4 years, respectively; \(p < 0.05\)).

Health data and questionnaires

Data about general health and lifestyle, sexual and reproductive health, previous or current (biochemical or clinical) pregnancies, ART procedures, and clinical diagnoses of the study subjects were obtained from the physicians and through gender-specific questionnaires administered to all study participants. All study subjects had been tested for STI (chlamydiosis, gonorrhoea, trichomoniasis, and mycoplasmosis) before commencing ART, therefore all recruited subjects were STI-free. None of the study subjects had received antimicrobial therapy within two months.

Nugent scoring of bacterial vaginosis

The diagnosis of bacterial vaginosis was based on the Nugent scoring system [16], using Gram stained slides of vaginal samples. Nugent score was evaluated on the basis of quantitative ratios of Lactobacillus, Gardnerella vaginalis, and Mobiluncus morphotypes seen in high-power magnification.

Semen sample assessment

Semen samples were obtained by masturbation and ejaculated into a sterile collection tube in a private room near the laboratories. The analysis of semen was performed according to WHO guidelines [17].
Level of leukocytes in seminal fluid (leukocytospermia) was determined in semen smears that were air-dried, Bryan-Leishman stained, and examined with the use of oil immersion microscopy. Significant level of leukocytospermia was estimated at ≥ one million leukocytes/ml of semen (WBC/ml) [17], whereas a lower threshold value (≥ 0.2 million WBC/ml) suggested by several recent studies [18, 19] was also used for comparison.

**ART**

Controlled ovarian stimulation was performed with recombinant FSH. Prevention of premature ovulation was performed with gonadotropin releasing hormone antagonist. Human chorionic gonadotropin was administered when two or more follicles reached the size of ≥ 17 mm in diameter and ovum pick-up (OPU) was performed 36 hours later. ART used in study couples was ICSI in 62% and IVF in the latter 38% of cases.

**Statistical methods**

The effect of various factors on the ART procedure was assessed using the logistic regression model, the odds ratio (OR), and 95% confidence interval (CI) were calculated using statistical software package STATA 12. For significance probability (p-value) calculation, Fisher’s exact test and Mann-Whitney U-test were used. Statistical significance was assumed at p < 0.05.

**Ethical Consideration**

Participation in the study was voluntary. Informed consent was obtained from all study subjects. The study was approved by the Ethics Review Committee on Human Research of the University of Tartu.

**Results**

Main study results are summarized in Table 1. Among the study cohort (100 couples), conception was achieved in and embryos were transferred to 96 female subjects. Thereafter, biochemical pregnancy was seen in 34 and clinical pregnancy in 29 females, thus achieving the IVF/ISCI success rate of 30.2%.

The present results showed that ART failure was moderately related to health problems and lifestyle factors, but significantly associated with female age (average 34.1 years, range 25-46) and excess body weight (22.4% of female subjects with BMI ≥ 25), although male subjects were significantly older than their female counterparts (average 34.1 vs. 37.4 years, p < 0.05) and 64.0% of men were overweight (BMI ≥ 25). In couples where male and female counterparts belonged to different age groups, with males being 36 years or older, the likelihood of conception was lower, but did not reach statistical significance.

Male factors, previous conceptions, and the presence of children increased the likelihood of IVF success (Table 1). The sperm quality did not affect ART result in the present subjects, however, decrease in sperm quality was observed in 61% of men with no children compared to 49% in men who had fathered 1 or more children.

Prevalence of bacterial vaginosis was 15.0% and that of prostatitis 7.1%, according to WHO standard (≥ one million leukocytes in one ml of seminal fluid) while 36.7% when

### Table 1. — Selected characteristics of the study subjects and their effect on IVF outcome.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Embryo transferred (total N=96)</th>
<th>IVF procedure success (N, %)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female study subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 25-35</td>
<td>55</td>
<td>23 (41.8%)</td>
<td>0.24 (0.09-0.66)</td>
<td>0.003</td>
</tr>
<tr>
<td>36+</td>
<td>41</td>
<td>6 (14.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI ≤ 24.9</td>
<td>73</td>
<td>28 (38.4%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt; 25.0</td>
<td>21</td>
<td>1 (4.8%)</td>
<td>0.08 (0.01-0.63)</td>
<td>0.002</td>
</tr>
<tr>
<td>Earlier miscarriages</td>
<td>28</td>
<td>9 (32.1%)</td>
<td>1.14 (0.44-2.94)</td>
<td>0.389</td>
</tr>
<tr>
<td>Earlier ectopic pregnancy</td>
<td>16</td>
<td>4 (25%)</td>
<td>0.73 (0.22-2.40)</td>
<td>0.432</td>
</tr>
<tr>
<td>Earlier abortions</td>
<td>23</td>
<td>10 (43.5%)</td>
<td>2.19 (0.82-5.80)</td>
<td>0.094</td>
</tr>
<tr>
<td>Previous deliveries</td>
<td>30</td>
<td>11 (36.7%)</td>
<td>1.54 (0.62-3.87)</td>
<td>0.413</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>29</td>
<td>11 (37.9%)</td>
<td>1.66 (0.66-4.19)</td>
<td>0.199</td>
</tr>
<tr>
<td>Tubal occlusion</td>
<td>27</td>
<td>10 (37.0%)</td>
<td>1.55 (0.60-3.97)</td>
<td>0.251</td>
</tr>
<tr>
<td>Absent fallopian tubes</td>
<td>19</td>
<td>5 (26.3%)</td>
<td>0.79 (0.25-2.44)</td>
<td>0.456</td>
</tr>
<tr>
<td>Salpingo-oophoritis</td>
<td>21</td>
<td>8 (39.1%)</td>
<td>1.58 (0.57-4.37)</td>
<td>0.263</td>
</tr>
<tr>
<td>Nugent score 0-6</td>
<td>81</td>
<td>25 (30.9%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nugent score 7+</td>
<td>15</td>
<td>4 (26.7%)</td>
<td>0.81 (0.24-2.81)</td>
<td>0.504</td>
</tr>
<tr>
<td><strong>Earlier pregnancies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>32</td>
<td>7 (21.9%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes, 1 to 3 times</td>
<td>58</td>
<td>19 (32.8%)</td>
<td>1.74 (0.64-4.74)</td>
<td>0.330</td>
</tr>
<tr>
<td>Yes, 4 or more</td>
<td>6</td>
<td>3 (50.0%)</td>
<td>3.57 (0.59-21.75)</td>
<td>0.255</td>
</tr>
<tr>
<td><strong>Earlier IVF experience</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>32</td>
<td>10 (31.3%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes, 1-3 times</td>
<td>46</td>
<td>16 (34.7%)</td>
<td>1.17 (0.44-3.07)</td>
<td>0.238</td>
</tr>
<tr>
<td>Yes, 4+ times</td>
<td>18</td>
<td>3 (16.6%)</td>
<td>0.44 (0.10-1.87)</td>
<td>0.134</td>
</tr>
<tr>
<td><strong>Leukocytospermia (M WBC/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 0.99</td>
<td>87</td>
<td>23 (26.4%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>≥ 1.00</td>
<td>7</td>
<td>4 (57.1%)</td>
<td>0.81 (0.24-2.81)</td>
<td>0.504</td>
</tr>
<tr>
<td><strong>Partner’s previous pregnancies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>39</td>
<td>7 (17.9%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes, with former partner</td>
<td>25</td>
<td>11 (37.9%)</td>
<td>2.31 (0.89-6.00)</td>
<td>0.070</td>
</tr>
<tr>
<td>Yes, with current partner</td>
<td>40</td>
<td>16 (55.2%)</td>
<td>2.21 (0.91-5.35)</td>
<td>0.062</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>54</td>
<td>11 (20.4%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes, with former partner</td>
<td>20</td>
<td>8 (27.6%)</td>
<td>1.75 (0.63-4.87)</td>
<td>0.210</td>
</tr>
<tr>
<td>Yes, with current partner</td>
<td>25</td>
<td>13 (44.8%)</td>
<td>3.72 (1.42-9.75)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Couples studied</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age both 25-35y</td>
<td>34</td>
<td>14 (41.2%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>one aged 36+</td>
<td>31</td>
<td>11 (35.5%)</td>
<td>0.79 (0.29-2.14)</td>
<td>0.415</td>
</tr>
<tr>
<td>both aged 36+</td>
<td>31</td>
<td>4 (12.9%)</td>
<td>0.21 (0.06-0.74)</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI both ≤ 24.9</td>
<td>27</td>
<td>11 (40.7%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>one ≥ 25.0</td>
<td>53</td>
<td>17 (32.1%)</td>
<td>0.69 (0.26-1.79)</td>
<td>0.300</td>
</tr>
<tr>
<td>both ≥ 25.0</td>
<td>14</td>
<td>1 (7.1%)</td>
<td>0.11 (0.01-0.98)</td>
<td>0.025</td>
</tr>
<tr>
<td>Smoking, neither</td>
<td>25</td>
<td>10 (40.0%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>one partner yes</td>
<td>44</td>
<td>14 (31.8%)</td>
<td>0.70 (0.25-1.94)</td>
<td>0.334</td>
</tr>
<tr>
<td>both partners yes</td>
<td>25</td>
<td>5 (20.0%)</td>
<td>0.38 (0.11-1.33)</td>
<td>0.108</td>
</tr>
</tbody>
</table>

1 Fisher’s exact test.
lower threshold value (≥ 0.2 M WBC/ml) from the authors’ previous studies [19] was applied. No statistically significant relationship was found in the present study between these conditions and IVF/ICSI outcome.

Discussion

In the present study of 100 consecutive infertile couples scheduled for ART procedure, pregnancy was achieved in 30.2% of the female subjects, with female age, overweight, and previous children from male side being the most significant factors affecting the ART outcome.

Importance of female age in achieving pregnancy has been repeatedly shown in other studies [3, 20], where higher age also indicated significantly lower ART success as well as increased probability for performing additional IVF/ICSI cycle(s). It has been estimated that every additional year of infertility decreases the likelihood of ART success by 2% [20].

Overweight is also a major risk factor of infertility [3, 4, 20, 21]. Overweight female subjects were found to have approximately half the chance of IVF/ICSI success when compared to females with normal weight [21]. Although the study group was relatively small, the chances of overweight females to conceive were still significantly lower (OR = 0.08; 95% CI 0.01–0.63; p = 0.002) in the present study.

Health problems described in subjects of this study were etiologically related to infertility as well as indications for ART procedure. Most prevalent findings in females were tubal factor infertility (52.0%), which was most often presented as partial or complete tubal occlusion (27.0%). Male subjects presented mostly with decline in sperm parameters (55.0%), where several problems (changes in sperm concentration, motility, and/or morphology) tended to present simultaneously.

Most subjects (67.0%) had a previous experience with ART procedure(s) and pregnancy was mostly (55.2%) achieved within three first cycles, which corresponds to previously published data [5]. It has also been pointed out that after the fourth IVF cycle, women with earlier IVF pregnancies had a significantly higher chance of conceiving than those who had not achieved any pregnancies during earlier IVF attempts [5]. This tendency could not be evaluated in the present study due to small study sample, however, the authors noted that history of earlier pregnancies and children still increased the likelihood of next pregnancy.

Smoking did not correlate with ART success in the present study, however, meta-analysis of 18 published studies where correlations between ART outcome and smoking were estimated, showed that smoking was a significant factor of IVF/ICSI failure in women, but not in men [22]. The present authors were unable to confirm alcohol consumption as a risk factor of IVF failure in this study, with similar results in a larger sample (221 couples) being published earlier [23].

One of the goals of this study was to estimate the prevalence of bacterial vaginosis and inflammatory prostatitis in ART subjects as well as to evaluate their correlation to ART outcome. Bacterial vaginosis (as measured by Nugent score) was present in 15% of women, but no correlation with ART outcome could be seen. Similar results have been reported in significantly larger study sample (307 patients) [12], although bacterial vaginosis prevalence was somewhat lower in that group (9.5%). However, in some studies bacterial vaginosis has been shown to be associated with early spontaneous abortion [24].

Inflammatory prostatitis also did not affect ART outcome in the present study, with similar results published in several earlier studies [14, 15], although some studies have shown possible negative effects of leukocytospermia on ICSI outcome [25, 26]. Number of couples where both counterparts were simultaneously affected by above-mentioned conditions was small, therefore possible negative effect on ART outcome could not be estimated.

The main drawback of this study was its small sample size, which also signified that the number of subjects with conditions or risk factors that could possibly interfere with ART outcome on a larger scale was very small in this study. Therefore, significantly larger study groups are required in further studies about correlations between health conditions and risk factors on one side and ART results on the other.

In conclusion, the most significant female factors affecting ART outcome are age and overweight, while previously fathered children is the most important factor in the male counterpart.

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References


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Comparative analysis of perinatal clinical problems in early and late preterm infants

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Summary

Objective: The aim of this study was to understand different clinical characteristics of early preterm infants (EPIs) and late preterm infants (LPIs). Materials and Methods: The clinical and laboratory data of 561 preterm infants, admitted to this hospital from January 2013 to December 2014, were comparatively analyzed. Results: EPIs accounted for 27.45% and LPIs accounted for 72.55%. The incidence rates of asphyxia at birth, placental abruption, and placenta previa in EPIs were significantly higher than those in LPIs (p < 0.01). The levels of albumin, globulin, triglycerides, serum phosphorus, serum iron, and hemoglobin in EPIs were significantly lower than those in LPIs (p < 0.01). Conclusions: LPIs accounted for the majority of preterm infants, placental abruption and placenta previa were the unique risk factors in EPIs, EPIs had lower nutritional reserves than LPIs, and would be more susceptible to the perinatal complications.

Key words: Early preterm infants; Late preterm infants; Perinatal period.

Introduction

Late preterm infants (LPIs) refer to infants with gestational age as 34~36 +6 weeks (with gestational age as 239~259 days after menstrua) [1-4], while early preterm infants (EPIs) refer to infants with gestational age as 28~33 +6 weeks (with gestational age as 197~238 days after menstrua). EPIs accounted for about 10%-13% of all hospitalized PIs [5-7], and LPIs accounted for majority of PIs. In recent years, with the development of assisted reproductive technology, and the increasing advanced maternal age, the proportion of EPIs exhibited a rising trend, while the exact demographic data of PIs are not known. Currently, the comparison between early term infants (with gestational age as 37~38 +6 weeks) and LPIs (with gestational age as 39~41 +6 weeks) has been more carefully assessed [8-10], while the comparison between EPIs and LPIs is rare. This study statistically analyzed the clinical data of EPIs and LPIs, such as perinatal factors, biochemical indicators, and various complications at birth, etc., aiming to explore the strategies to further reduce mortality rate and improve clinical outcomes.

Materials and Methods

Study subjects

The study included 561 PIs, born and admitted into neonatal unit of the present hospital from January 2013 to December 2014, which were selected, including 154 EPIs and 407 LPIs. The infants that were discharged while re-hospitalized for other diseases were not included in this study. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Maternal and Child Health Hospital of Yiwu. Written informed consent was obtained from all participants’ guardians.

Methods

The clinical data of PIs admitted into the present department: were retrospectively collected, including gender, gestational age, birth weight, delivery mode, maternal pregnancy complications (including hypertension, anemia, diabetes, cholestasis), obstetric factors (including birth asphyxia, premature rupture of membrane, placental abruption, placenta previa, abnormal amniotic fluid), and twin or embryo transplantation pregnancy. The clinical data of LPIs and EPIs were comparatively studied. One hour after PIs admitted into the present department, radial artery blood was sampled under aseptic conditions for biochemical blood tests and blood routine tests. The diagnostic criteria of other complications were referred by “practical neonatology”, fourth edition [12].
Table 1. — Comparison of preterm indicators [n (%)].

<table>
<thead>
<tr>
<th>Obstetric indicator</th>
<th>EPIs (n=154)</th>
<th>LPIs (n=407)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin</td>
<td>24 (15.58)</td>
<td>58 (14.25)</td>
<td>0.16</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>C-section</td>
<td>63 (40.91)</td>
<td>196 (48.16)</td>
<td>2.36</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Asphyxia at birth</td>
<td>68 (44.16)</td>
<td>37 (9.09)</td>
<td>90.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maternal hypertension</td>
<td>10 (6.49)</td>
<td>43 (10.57)</td>
<td>2.17</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Maternal anemia</td>
<td>6 (3.90)</td>
<td>6 (1.47)</td>
<td>2.08</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Maternal diabetics</td>
<td>5 (3.25)</td>
<td>6 (1.47)</td>
<td>1.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Maternal cholestasis</td>
<td>11 (7.14)</td>
<td>32 (7.86)</td>
<td>0.08</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>38 (24.68)</td>
<td>131 (32.19)</td>
<td>2.99</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>12 (7.79)</td>
<td>7 (1.72)</td>
<td>12.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Abnormal amniotic fluid</td>
<td>24 (15.58)</td>
<td>42 (10.32)</td>
<td>2.98</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Embryo transplantation</td>
<td>10 (6.49)</td>
<td>19 (4.67)</td>
<td>0.76</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 2. — Comparison of blood biochemistry (±s).

<table>
<thead>
<tr>
<th>Blood biochemistry</th>
<th>EPIs (n=154)</th>
<th>LPIs (n=407)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/L)</td>
<td>33.72±4.40</td>
<td>35.92±3.06</td>
<td>6.68</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>14.31±3.57</td>
<td>15.39±4.34</td>
<td>2.76</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total bilirubin (μmol/L)</td>
<td>12.38±9.54</td>
<td>13.12±11.59</td>
<td>0.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.45±0.49</td>
<td>0.62±0.46</td>
<td>3.84</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.46±0.72</td>
<td>2.32±0.81</td>
<td>1.88</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total calcium (mmol/L)</td>
<td>2.23±0.19</td>
<td>2.25±0.23</td>
<td>0.96</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>1.88±0.52</td>
<td>2.17±0.58</td>
<td>5.43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum iron (μmol/L)</td>
<td>14.52±6.19</td>
<td>17.62±7.99</td>
<td>4.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>162.85±22.13</td>
<td>170.28±20.83</td>
<td>3.71</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 3. — Comparison of birth complications [n (%)].

<table>
<thead>
<tr>
<th>Complication</th>
<th>EPIs (n=154)</th>
<th>LPIs (n=407)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaundice</td>
<td>144 (93.51)</td>
<td>366 (89.93)</td>
<td>1.73</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>27 (17.53)</td>
<td>21 (5.16)</td>
<td>21.86</td>
<td>&lt;0.01</td>
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<tr>
<td>Hypoglycemia</td>
<td>34 (22.08)</td>
<td>47 (11.55)</td>
<td>10.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Transient tachypnea</td>
<td>31 (20.13)</td>
<td>97 (23.83)</td>
<td>0.87</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>50 (32.47)</td>
<td>22 (5.41)</td>
<td>73.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apnea</td>
<td>57 (37.01)</td>
<td>28 (6.88)</td>
<td>78.91</td>
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</tr>
<tr>
<td>Feeding intolerance</td>
<td>46 (29.87)</td>
<td>20 (4.91)</td>
<td>67.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Assisted ventilation</td>
<td>45 (29.22)</td>
<td>19 (4.67)</td>
<td>66.64</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Results

Basic conditions

The EPIs group included 86 males (55.84%) and 68 females (44.16%), with gestational age of 30.47 ± 1.52 weeks, birth weight as 1,702.62 ± 494.93 grams. The LPIs group included 252 males (61.92%) and 155 females (38.08%), with gestational age of 35.23 ± 0.94 weeks, and birth weight as 2,707.07 ± 473.66 grams. The gender ratio showed no statistical significance between the two groups (χ² = 44.57, p > 0.05; the gestational age and birth weight of the EPIs group was significantly lower than the LPIs group, and the difference was statistically significant (t = 44.57, 22.14, p < 0.01).

The incidence rates of asphyxia at birth, placental abruption, and placenta previa in the EPIs group were significantly higher than the LPIs group, and the differences were statistically significant (p < 0.01, Table 1). Albumin, globulin, triglycerides, serum phosphorus, serum iron, and hemoglobin level in the EPIs group were significantly lower than the LPIs group, and the differences were statistically significant (p < 0.01, Table 2). Hypothermia, hypoglycemia, respiratory distress, apnea, feeding intolerance, and assisted ventilation rate in the EPIs group were significantly higher than the LPIs group, and the differences were statistically significant (p < 0.01, Table 3).

Discussion

Overview of preterm birth

Preterm birth was a leading cause of neonatal death and long-term neurological sequelae. In the past ten years, the preterm birth rate has increased by 33% in USA from 1981 to 2006, and the mortality rate was higher [2], among which LPIs accounted for 70% of all PIs, and the increased birth rate of LPIs was the main reason of the increasing of PIs [13]. The data showed that LPIs accounted for 72.55% of all PIs, and a survey of birth status in urban China in 2005 showed that PIs with gestational age as 32 to 36 weeks, accounted for 85.2% of all PIs [14]. The recent increasing of PIs had several reasons, among which the frequently reported ones included maternal demographic changes (such as late childbearing), infertility treatment, increased maternal age, multiple pregnancy, and pregnancy-concomitant diseases, especially obesity [15, 16]. The statistics confirmed that maternal chorioamnionitis, high blood pressure, and premature rupture of membranes could cause later preterm birth [17]. The previous history of premature birth, too short interval between two pregnancies (<12 months), and bleeding in early pregnancy increased the risk of preterm birth [17].

Features of PIs after delivery

1) Appearance included relatively large head, accounting for one-third of body, wider fontanelle, softer skull and nails, fluffy hair, undescended or non-fully descended testes in males, the labium minus pudenda was not covered by labium major pudenda in females. 2) Thermoregulation included: lacking mature development of body temperature center, less subcutaneous fats, while larger surface area, less muscle activities and autologous heat production, and
with easy body heat loss. Therefore, under normal circumstances, the body temperature would decrease in lower ambient cold conditions, which might even cause intracranial hemorrhage in severe cases. 3) Nervous system included poorer nerve reflexes and the infant would usually be in the sleeping state. If the body weight was 1,500 grams or less, infants would also be prone to intracranial hemorrhage, requiring extensive clinical attention. 4) Immune functions, compared with the full-term infants, PIs had poorer immune function lacking adequate anti-scavenging abilities against bacteria and viruses, and could only acquire less immunoglobulins from mother. Because they had weak resistance to infections, therefore, they were prone to sepsis, which might ultimately enhance the mortality rate to a larger extent.

**Predisposing factor analysis of EPIs and LPIs**

The risk factors of preterm birth reported included threatened eclampsia, placental abruption, intrauterine fetal growth restriction, and other adverse signs, oligohydramnios, pre-pregnancy diabetes and gestational diabetes, and abnormal fetal heart rate, etc; however, 6.1%~23.2% of PI showed no preterm signs [1]. The data of this study showed that the gender ratio between the two groups showed no statistically significant difference ($\chi^2=1.72, p > 0.05$), gestational age and birth weight of EPIs were significantly lower than LPIs, and the differences were statistically significant ($t = 44.57, 22.14, p < 0.01$), the incidence rates of asphyxia at birth, placental abruption, and placenta previa of EPIs were significantly higher than LPIs, and the differences were statistically significant ($p < 0.01$), indicating that placenta previa and placental abruption were the unique risk factors for EPIs. Therefore, high-risk factors for EPIs should be especially considered, to reduce the incidence rate of LPIs, which is also significant to reduce neonatal mortality.

**Effects of preterm birth on biochemical indicators of EPIs and LPIs**

In order to improve the chances of survival, EPIs would have a series of adaptive changes in utero, including intrauterine growth and metabolism. After delivery, detached from the adverse intrauterine environments, growth and metabolism of EPIs would be different from LPIs. The total protein in EPIs could sensitively react to the changes of nutritional status and it was the important indicator to evaluate the nutritional status and detect the effects of nutritional support internationally [18]. The data showed that the levels of albumin, globulin, triglycerides, serum phosphorus, serum iron, and hemoglobin in EPIs were significantly lower than LPIs, and the differences were statistically significant ($p < 0.01$), indicating that EPIs would be more prone to anemia, infections, and other diseases, so a more active and reasonable nutritional support would be required.

**Effects of preterm birth on complications of EPIs and LPIs**

Foreign large numbers of clinical studies have found that the common clinical problems in PIs included NRDS, neonatal transient dyspnea, hyperbilirubinemia, feeding difficulties, and low blood sugar, and among which the most common was respiratory diseases [19]. The data showed that hypothermia, hypoglycemia, respiratory distress, apnea, feeding intolerance, and assisted ventilation rate in EPIs were significantly higher than LPIs, and the differences were statistically significant ($p < 0.01$), consistent with the fact that respiratory distress was the most prominent clinical problem. In addition, placenta previa, placental abruption, as well as such intrauterine fetal blood loss due to fetal maternal blood transfusion and twin blood transfusion, could cause fetal anemia, maternal hypertension, and other vascular diseases, thus resulting in small fetal development, hypoxia, etc., which required more clinical attention [19].

In conclusion, LPIs accounted for the majority of PIs, and placental abruption and placenta previa were the unique risk factors in EPIs. EPIs had lower nutritional reserves than LPIs and would be more susceptible to perinatal complications.

**References**


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Introduction

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of variable severity, occurring or being detected for the first time during pregnancy [1]. Nearly 1% to 14% of all pregnancies (average 7%) are complicated by gestational diabetes mellitus (GDM) [2], with a strong correlation between metabolic control and feto-maternal outcomes [3, 4]. Maternal hyperglycemia has been shown to be a significant risk factor for the mother and fetus [5]. Failure to inadequately diagnose and treat this condition may lead to significant perinatal mortality and morbidity, including stillbirths, fetal macrosomia, shoulder dystocia, birth trauma, respiratory distress syndrome, neonatal jaundice, neonatal electrolyte imbalance, and polycythemia [6]. In addition, babies born to mothers with GDM are more likely to experience health problems such as hypoglycemia, hypocalcemia, hypomagnesemia, hyperbilirubinemia, and respiratory distress syndrome (RDS) during the newborn period [7]. After confirmation of the positive effects of optimally controlled GDM on perinatal outcomes in recent studies, the discussions concerning the importance of screening and treatment in this condition have subsided [8]. Current strategy for the prevention of fetal and maternal complications involves the assessment of all pregnant women between 24 and 28 weeks of gestation with a 50-gram oral glucose challenge test (GCT). Pregnant women with a test result of > 140 mg/dl are subjected to further assessment with oral glucose tolerance test (OGTT) [9], which allows the establishment of a final diagnosis [10]. A single value exceeding the normal range in OGTT is referred to as “glucose intolerance” or “borderline diabetes” [11] while two or more readings above the normal range are diagnostic for GDM. Pregnant women diagnosed with GDM are treated accordingly (diet, insulin, etc.) to prevent obstetric complications and postpartum type 2 diabetes [12].

Although appropriate medical care is generally provided for pregnant women with GDM due to its known effects on both the fetus and the mother, the clinical importance of a single high reading in a three-hour (100 grams) OGTT is unknown. Many studies have shown that single high reading in OGTT is a common occurrence with potential adverse feto-maternal effects [13].

In the present study, the authors’ objective was to examine perinatal outcomes in that specific subset of pregnant women in whom a diagnosis of GDM could not be established, but in whom OGTT was abnormal.

Materials and Methods

This study was approved by the local ethics committee at Sifa University and written consent of the participating patients were obtained. The study design was a cross-sectional study was performed on 200 pregnant women who presented to the antenatal outpatient unit of the Sifa University, Department of Gynecology and Obstetrics between January 2012 to November 2014. Patients with a previous diagnosis of diabetes, metabolic disease, or multiple-pregnancy were excluded. A standard screening test with 50 grams of glucose was administered to all study subjects Plasma glucose
levels were measured spectrophotometrically with a device using the hexokinase method. Patients with a blood glucose level <140 mg/dl were classified as “normal”, while those with > 200 mg/dl were considered to have “GDM” and those with a glucose >140 mg were considered to have “impaired glucose tolerance (IGT)”. The latter group subsequently underwent a 100-gram OGTT after eight to 14 hours of fasting. Prior to OGTT, pregnant women were instructed to consume a diet that included a minimum of 150 grams of carbohydrates for three days and the test was performed in the morning following eight to 12 hours of overnight fasting. After blood sampling for fasting blood glucose determination, a solution containing 100 grams of glucose was given to the study subjects and one-, two-, and three-hour venous glucose levels were assessed according to Carpenter and Coustan’s threshold criteria, where two or more readings above the normal range were considered GDM, and one reading was considered IGT. Patients with normal glucose readings at all time-points were accepted to have “normoglycemia”.

Study subjects were divided into two groups based on the result of OGTT. Group A (n=21) included patients with a single abnormal reading at OGTT (i.e. IGT group), and Group B (n=28) included women with gestational diabetes (i.e. GDM group). Gestational weeks, glucose concentrations measured during a three-hour OGTT, BMI, age, parity, birth weight, the birth method, and maternal and perinatal morbidities were assessed and recorded. All births took place in the hospital setting at the Private Sifa Hospital.

In patients diagnosed with GDM, insulin therapy was initiated when dietary treatment did not consistently maintain fasting and preprandial capillary glucose ≤ 100 mg/dl and two-hour post-prandial capillary glucose ≤ 120 mg/dl. Patients were closely monitored for pregnancy related complications.

Pre-eclampsia was defined as persistently high blood pressure (systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg in > two measurements) and presence of proteinuria (urinary protein >+2), and pregnancy-induced hypertension was defined according to Carpenter and Coustan’s threshold criteria without proteinuria. Birth before 37 weeks of gestation, term-induced births, fetal membrane rupture with amniotomy, or administration of intravenous oxytocin infusion was considered “pre-term”.

Macrosomia was defined as a birth weight exceeding 4,000 g and neonatal hypoglycemia was defined as the occurrence of a plasma glucose level of < 40 mg/dl in the first 48 hours of life [14].

Presence of a respiratory rate above 60/minute in addition to clinical findings such as subcostal retractions, grunting, and nasal flaring were considered to indicate respiratory distress. In addition, patients with lung x-ray findings suggestive of respiratory distress syndrome (RDS), pneumonic infiltration or pneumothorax were recorded.

Direct and indirect hyperbilirubinemia were measured with an auto-analyzer in venous blood samples and phototherapy was administered according to the protocol for admitted newborns with a gestational age of ≥35 weeks proposed by the Hyperbilirubinemia Subcommittee of the American Academy of Pediatrics [15].

Results

The demographic characteristics of the groups are shown in Table 1. No significant differences were observed in BMI, parity, and age between the groups. There were no significant differences between IGT and GDM groups in terms of FBG, while significantly higher blood glucose was found at GCT and at all OGTT time-points (one, two, and three hours) in GDM group (Table 1).

The groups were also compared in terms of obstetric outcomes (Table 2), with no significant differences (p > 0.05) in the frequency of preterm labour (PL), pregnancy induced hypertension (PIH), pre-eclampsia, polyhydramnios, or macrosomia. However, significantly higher fetal birth weight as well as a higher occurrence of cesarean deliveries were found in GDM group (Tables 1 and 2) (p < 0.05).

Neonatal outcomes were compared between the two groups (Table 2), with no significant differences in the frequency of hyperbilirubinemia, hypoglycemia or RDS (p > 0.05) (Table 2).

Discussion

Although numerous studies on GDM have been published since its first description, the clinical significance of the milder form of the condition remains disputed, with no agreement on screening tests, diagnostic criteria, and use of oral anti-diabetics [16].

A 50-gram oral glucose challenge test may be used irrespective of the fasting state between 24 and 28 weeks of gestation in all pregnant women, although tests performed during fasting state are diagnostically more sensitive [17]. American Diabetes Association (ADA) accepts a threshold...
level of 130 mg/dl or 140 mg/l, which allows identification of the 80% or 90% of the cases, respectively [18].

From a viewpoint of patient characteristics, advanced age, increased BMI, parity, and fetal macrosomia are more prevalent in GDM patients [19, 20]. However, in the present study no significant differences in terms of age, parity, or BMI was found between the groups. On the other hand, consistent with previous studies, a parallel increase in plasma glucose and birth weight was observed [21].

Similar to a previous study [22], the risk of cesarean delivery was also high in the present patients with GDM, which could be associated with an increased likelihood of birth trauma, fetal distress, or postpartum bleeding in women with GDM. In contrast, IGT could be associated with a lower potential for planned cesarean delivery. In patients with borderline GDM, a higher frequency of amniotic fluid index exceeding 95-97.5 percentile was found [23]. However, the present study groups did not differ in this respect. Insulin resistance has been shown to be associated with the development of pre-eclampsia [24]. In this study, despite the absence of a significant threshold level, an increased risk of PIH and pre-eclampsia was found in the study group. This may be due to the small sample size. In contrast to other reports [25], no significant increase in preterm labor was found in association with GDM.

In a study by Sermer et al. where OGTT results of 3,637 pregnant women was examined, a linear correlation between increasing glucose levels and hyperbilirubinemia was observed [26]. In the present study, 25% and 19% of the newborns in GDM and IGT groups had hyperbilirubinemia, respectively, which was treated with phototherapy.

The leading cause of mortality in newborns of diabetic mothers is RDS, which results from fetal hyperinsulinism inhibiting the synthesis of surfactant in the fetal lung. In the study by Casey et al., 3% of the 874 pregnant women with GDM had RDS [27]. In the present study, RDS occurred in 21% and 14% of the newborns of mothers in GDM and IGT groups, respectively. A major limitation of this study is the small sample size. Thus, further studies with larger sample sizes are warranted.

In conclusion, the present results suggest that single high glucose readings in OGTT may be as important as a diagnosis of GDM in terms of fetomaternal complication risk. Well-designed, larger prospective studies involving borderline GDM patients are warranted to further clarify this association.

### References


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Evaluation of ten years of intrauterine insemination results at a tertiary center

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¹ Süleymaniye Maternity Research and Training Hospital, Istanbul; ² Haseki Research and Training Hospital, Istanbul (Turkey)

Summary
Purpose: To report on ten years of intrauterine insemination (IUI) practice at Haseki Training and Research Hospital to determine retrospectively, the impact of IUI on the management of subfertile couples. Materials and Methods: This study was a retrospective analysis of all IUI cycles completed from June 1, 2003, to July 1, 2013, at the Haseki Training and Research Hospital, Istanbul, Turkey. Baseline clinical characteristics, drugs used for ovulation induction, and triggering ovulation were reviewed. The primary outcome was clinical pregnancy. Results: The overall clinical pregnancy rate was 10.2% (26/253). Improved success was significantly associated with a shorter period of infertility (4.8 ± 3.9 years vs 3.2 ± 2.4 years; p = 0.01). Clinical pregnancy rates were significantly higher when recombinant FSH was used for ovulation induction than clomiphene citrate (CC) (22% vs 5.6%; p = 0.002). Patients were also analysed for the drug used for triggering ovulation. The clinical pregnancy rate was 27.2% in the recombinant hCG group compared with 8.6% in the urinary hCG group (p = 0.006). Conclusions: IUI may be a safe and cost-effective option for mild male factor infertility or unexplained infertility. Better results may be obtained when recombinant FSH and recombinant hCG are used and when the duration of infertility is short.

Key words: Intrauterine insemination; Clinical pregnancy rate; Ovulation induction; Ovulation triggering; Unexplained infertility.

Introduction
Intrauterine insemination (IUI) is an assisted reproduction procedure that places sperm directly into the uterus. This method is indicated in cases of cervical infertility, relative male factor infertility, anovulation, mild endometriosis, and unexplained infertility [1]. Ovulation induction with clomiphene citrate (CC) prior to IUI is commonly recommended in couples, especially with unexplained infertility. More commonly, gonadotropin therapy is combined with IUI for the treatment of unexplained infertility. IUI involves timed insemination of spermatozoa into the uterus in natural cycles or insemination following stimulation of the ovaries using CC or gonadotropins [2]. It is modestly effective and reasonable to consider in couples who fail to conceive during IUI cycles combined with clomiphene [3]. Controlled ovarian hyperstimulation is a technique commonly utilized with assisted reproductive technologies. It has the advantage of increasing the number of oocytes available for fertilization, thus improving pregnancy rates per stimulated cycle [4].

The aim of this retrospective study was to report on ten years of IUI practice at Haseki Training and Research Hospital to determine the impact of IUI on the management of subfertile couples with regards to success rate and to identify prognostic factors associated with successful outcome.

Materials and Methods
The authors analysed all IUI cycles completed from June 1, 2003, to July 1, 2013, at the Haseki Training and Research Hospital, Istanbul, Turkey. All couples had unexplained or male factor infertility and had been referred to the infertility clinic. Unexplained infertility was defined if no abnormality was found during primary infertility investigations with regards to ovarian, tubal, uterine, and male factors. The inclusion criteria of this study were as follows: female aged between 20 and 45 years, unexplained infertility for at least 12 months, and patent fallopian tubes documented by hysterosalpingography. Patients who had endometriosis (classification stage III and IV of the American Infertility Society) [5], contraindication to one of the used drugs, persistent ovarian cyst (a cyst of at least 30 mm persisting for longer than two months), and having a total motile sperm count less than a million in prepared semen, were excluded from the study.

All participants underwent a baseline transvaginal ultrasound using a 7.5-MH transvaginal probe on the second or third day of the menstrual cycle to rule out the presence of an ovarian cyst.

Ovarian stimulation
All women in their first ovulation induction/IUI cycle underwent transvaginal ultrasound monitoring between cycle days 11 and 13 after receiving five days of treatment with 100 mg of CC daily starting on days 3 to 5. At the time of the ultrasound, the mean diameter of the follicle was calculated from measurements in two perpendicular planes for any follicles measuring greater than 16 mm. If at least one follicle ≥ 18 mm in mean diameter was detected, hCG was administered and IUI was scheduled 36 hours later. If follicular maturation was not successful with CC, these women underwent ovulation induction with gonadotropins.

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The treatment was initiated on the third day of the cycle and was continued until ovulation. The authors used recombinant FSH. The administration of GnRH agonist or antagonist was not required. The initial dose of gonadotropin prescribed (37.5-100 IU/day) depended on the woman’s hormonal profile, age, and the duration of infertility. The initial dose was maintained until the first sixth day of stimulation and thereafter adapted as a function of the ovarian response.

To evaluate the patients, the authors used vaginal ultrasound examination evaluating follicle number and size and sperm parameters, ovulation triggering was achieved by SC injection of 250 mcg of recombinant hCG or 10,000 IU u-hCG. Insemination was performed 36 hours after the hCG injection. The criteria used for triggering ovulation was at least one follicle measuring ≥ 18 mm. Participants did not receive luteal phase support.

**IUI**

Semen specimens were produced by masturbation at the laboratory, following 48-72 hours of abstinence, two hours before the insemination. After a motility determination, the spermatozoa were washed free from seminal liquid and prepared for insemination (postwash TMS - number of spermatozoa inseminated). Abnormal spermatozoa were rated according to the WHO criteria [6].

A soft catheter was used for the insemination process. The end of the catheter was placed in the center of the uterine cavity, and the sperm preparation (0.2-0.4 ml) was injected slowly (over 20 seconds). The authors did not use progesterone therapy after insemination. After insemination, the woman was allowed to perform a pregnancy test (serum beta-hCG assay). If the test was positive, it was repeated seven days later to check the beta-hCG time course. The pregnancy was qualified as ongoing when it reached 12 weeks.

Numerical variables were reported as mean ± standard deviation (SD) when normally distributed, otherwise as median plus range. All variables were tested for normal distribution with Kolmogorov-Smirnov test. Continuous variables were compared with the t-test for independent samples on the Mann-Whitney U-test depending on the normality of their distribution. Proportions were compared with the Fischer’s exact test or the chi-squared test where appropriate. A p-value ≤ 0.05 was considered statistically significant. All analyses were performed using SPSS version 21.0. The endpoints were clinical pregnancy rate (defined as ultrasound evidence of pregnancy) per cycle. The study protocol was approved by the local independent ethics committee.

**Results**

The authors studied a total of 253 IUI cycles in 235 couples; 235 couples underwent one IUI cycle and 18 couples underwent two IUI cycles. Of these patients, 159 received CC and 94 received recombinant FSH for ovulation stimulation. Descriptive summaries of all the patients can be seen in Table 1.

The group treated with recombinant FSH was found to have significantly higher pregnancy rates. There was a difference in pregnancy outcomes between the two groups. Higher pregnancy rates were achieved with recombinant FSH in comparison with CC. Cycles of ovulation induction

<table>
<thead>
<tr>
<th>Table 1. — Baseline characteristics of all patients.</th>
</tr>
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<tbody>
<tr>
<td><strong>Median</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
</tr>
<tr>
<td>Cause of infertility:</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Primary infertility</td>
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<tr>
<td>Drug used for ovulation induction:</td>
</tr>
<tr>
<td>CC</td>
</tr>
<tr>
<td>Recombinant FSH</td>
</tr>
<tr>
<td>Drug used for triggering ovulation:</td>
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<td>Choriogonadotropin</td>
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<tr>
<td>Choriogonadotropin alfa</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table 2. — Factors affecting pregnancy rates in intrauterine insemination.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
</tr>
<tr>
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</tr>
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<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Infertility duration</td>
</tr>
<tr>
<td>Cause of infertility:</td>
</tr>
<tr>
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</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Type of infertility:</td>
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<td>Primer</td>
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<tr>
<td>Seconder</td>
</tr>
<tr>
<td>Drug used for ovulation induction:</td>
</tr>
<tr>
<td>CC</td>
</tr>
<tr>
<td>Recombinant FSH</td>
</tr>
<tr>
<td>Drug used for triggering ovulation:</td>
</tr>
<tr>
<td>u-hCG</td>
</tr>
<tr>
<td>Recombinant hCG</td>
</tr>
<tr>
<td>Leading follicle diameter at the time of hCG (mm)</td>
</tr>
<tr>
<td>Number of follicles</td>
</tr>
<tr>
<td>Sperm count (mil/ml)</td>
</tr>
<tr>
<td>Sperm motility</td>
</tr>
<tr>
<td>Sperm morphology</td>
</tr>
</tbody>
</table>
with CC had a 5.6% pregnancy rate, whereas cycles with recombinant FSH had a 22% pregnancy rate (p = 0.002). There was no statistical significant difference in pregnancy rates according to patient’s age, body mass index or type of infertility (Table 2). Duration of infertility was significantly shorter for the pregnant group than non-pregnant group. (4.8 vs 3.2, p = 0.01)

When pregnancy rates were compared to the drug used for ovulation induction, pregnancy rates were significantly higher with recombinant hCG group than u-hCG group (27.2% vs 8.6% respectively, p = 0.006). When comparing the cycles that resulted in pregnancy versus those that did not, there was no statistical significant difference in the sperm count, motility, and morphology of the males.

Discussion

In this study, the authors found that duration of infertility, drug used for ovulation induction and drug used for triggering ovulation significantly affects the pregnancy rates after IUI. The overall pregnancy rate was found as 10.2% and this result was similar to other studies. Wainer et al. reported the pregnancy rate as 12.91%, Iberico et al. reported the pregnancy rate as 9.2% per cycle [7, 8]. Consequently, the present authors decided to evaluate their results and search for prognostic factors associated with successful outcome after IUI.

Predictors of IUI cycle success in achieving pregnancy have been examined by several studies [9-12]. Factors that were considered to be prognostic included the age of patients, duration of the infertility, primary infertility diagnosis, number of mature follicles, and sperm parameters. In this study, the authors examined the optimal follicular size before hCG administration and drug used to trigger ovulation to yield the highest pregnancy rates in IUI cycles. In both groups, the diameter of the leading follicle at the time of hCG administration was not statistically different (19.9 vs 20.6 mm); however, when the patients were evaluated according to drug used to trigger the ovulation, there was a statistically significant difference favoring recombinant hCG when compared with u-hCG (27.2% vs 8.6% respectively, p = 0.006). Similarly Abdelmassih et al. found a higher frequency of positive beta hCG values and clinical pregnancy rate in the recombinant hCG group, but their differences did not reach statistical significance [13]. Hugues et al. also reported the recombinant hCG products ensure a better hormonal environment during the luteal phase and thought to be related with better pregnancy results [14].

In the present study, the most important step for pregnancy occurrence was the drug used for ovulation induction. Cycles of ovulation induction with CC had a 5.6% pregnancy rate, whereas cycles with recombinant FSH had a 22% pregnancy rate (p = 0.002). Similarly Hughes analyzed the data from 22 studies and a total of 5,214 cycles [15]. The pregnancy rate per cycle for unexplained infertility was 15% for stimulation with gonadotropin, 6% in natural cycles, and 7% for stimulation with CC. Also Guzyck et al. published a meta-analysis of 45 studies and 1,806 IUI cycles [16]. The pregnancy rates in natural cycles and those stimulated with CC or hMG were 3.8% (n=378), 7.7% (n=315), and 17.1% (n=1113), respectively.

Another important factor for clinical pregnancy after IUI was found to be duration of infertility. Nuojua-Huttunen et al. reported significantly different pregnancy rates according to whether the length of infertility was below or above six years [17]. Similarly the present authors also found that duration of infertility, independently from the drug used for ovulation induction, significantly influenced the pregnancy rate. Women who became pregnant had significantly shorter infertility duration than women who did not (4.8 vs 3.2 years, p = 0.01).

In this study BMI and type of infertility (primer or seconder) did not appear to affect pregnancy rates, and Dodson and Legros did not find any difference, although they observed that the dose of gonadotropin had to be increased for stimulation in obese women [18].

Sperm parameters may also play an important role in the success of IUI. Sakhel et al. obtained a pregnancy rate per cycle of 30.3% with more than five million spermatozoa/ml vs 18.8% with less than five million (p = 0.1) Belaisch-Allart et al. obtained a pregnancy rate per cycle of 12.5% with less than ten million spermatozoa/ml and 17% with more than 20 million. This difference was not statistically significant but illustrates a relationship between number of spermatozoa and the pregnancy rate [19]. In the present study, sperm count was higher in the pregnant group but this was not statistically significant (35.9 vs 31.7 million/ml) Sperm motility and morphology were not found to be correlated with pregnancy. A possible explanation for sperm motility and morphology not being a predictive factor of the pregnancy outcome may be because the majority of men in the present study couples had total motile sperm count above threshold.

Goverde et al. stated that the woman’s age is the most important factor influencing the likelihood of pregnancy, whatever treatment is chosen (IUI or IVF) [20]. In contrast, Brezeciffa et al. reported that the age of women under 40 years had no influence on the pregnancy rate after stimulation with clomiphene citrate and hMG [21]. Similarly the present authors found that patients who became pregnant after IUI were younger, but this did not reach statistical significance (28.5 vs 29.7, respectively). It may be because most of the patients were younger than 40 years.

Silverberg et al. [22] analyzed IUI cycles after ovulation induction with human menopausal gonadotropins and found that ovulation success was higher in follicles larger than 20 mm on the day of hCG administration, although there was no relationship between the size of the leading follicle and cycle outcome. Also, Iberico et al. [8] found higher pregnancy rates when leading follicle was larger...
than 20 mm, but this finding was not statistically significant. Similarly the present authors also did not find a relationship for pregnancy rates for the leading follicle size.

In conclusion the present authors found that shorter duration of infertility is significantly associated with higher pregnancy rates. Also, drug used for ovulation induction and drug used for triggering ovulation significantly affects the pregnancy rates after IUI. Although the present authors analysed patients for ten years, retrospectively, a larger sample size may help in formulating better predictive parameters for IUI success. This is the limitation of this study, but all IUI procedures have been administered in a uniform manner by a unique team at a tertiary center. Since IUI represents a cost-effective and safe treatment of subfertility, it may enable the early identification of couples who would probably benefit from in vitro fertilization.

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Introduction

Placenta previa is an obstetric complication where the placenta is inserted into the lower uterine segment, adjoining or covering the cervical os. Placenta accreta, which involves abnormal adherence of the placenta to the myometrium, is a serious complication of placenta previa [1, 2]. A well-known risk factor for placenta accreta is previous cesarean delivery complicated by placenta previa. In addition, no reports have specifically detailed an increased risk of placenta accreta in pregnant women with a history of adenomyomectomy.

The present authors report a case of placenta accreta in a woman who had previously undergone laparoscopic adenomyomectomy. Consequently, delivery was by cesarean section and hysterectomy. They also present the relevant details of the case and discuss the implications for the management of pregnancy following laparoscopic adenomyomectomy.

Case Report

A 41-year-old female (gravida 5, para 0, spontaneous abortion 3, dilation and curettage 1) was referred to the present hospital at 31 weeks of gestation with complete placenta previa. She had presented with vaginal bleeding at 30 weeks of gestation, at which point she had been admitted to another hospital. Six years earlier, she had undergone ovarian cystectomy and laparoscopic adenomyomectomy of the posterior uterine wall for endometriosis and dysmenorrhea. The entire posterior uterine wall had been resected and repaired by laparoscopic suture, and no myometrial defects were noted at the conclusion of the procedure.

In the current pregnancy, the placenta was located anterior-to-posterior deep in the uterine wall, with suspected complete placenta previa. Ultrasonography revealed only two small lacunae, suggesting a low risk of placenta accreta (Figure 1A). Fetal growth was appropriate for the gestational age. The patient was considered at low risk for placenta accreta and high risk for complications due to the total placenta previa. To address the small possibility of placenta accreta, a multidisciplinary team (obstetrics, perinatology, gynecology, urology, and interventional radiology) was assembled, and cross-matched blood was available at all times during her hospitalization.

At 34 weeks and four days of gestation, the patient experienced a sudden onset of vaginal bleeding. Because the bleeding continued, with a total blood loss of approximately 500 ml, the authors performed an emergency cesarean section under general anesthesia. A laparotomy showed that the placenta and multiple small blood vessels were located in the lower uterine segment. They also performed a vertical uterine incision to prevent an incision through the placenta and successfully delivered a healthy male infant weighing 2,542 grams, with Apgar scores of 8 and 9 at one and five minutes, respectively. However, the placenta was not spontaneously delivered. Because of the abnormal uterine adherence and continued bleeding from the placental site, the authors performed a conventional supracervical hysterectomy with the placenta in situ. The procedure was unremarkable except for injury to the bladder, which was repaired by an urologist. Total blood loss was approximately 3,000 ml, with the patient receiving a 2,240 ml transfusion of

Summary

Background: The influence of adenomyomectomy on subsequent pregnancy is unknown. Placenta accreta is most often associated with placenta previa in women with multiple previous cesarean sections. Case: A 41-year-old woman became pregnant six years after a laparoscopic uterine posterior adenomyomectomy. She was diagnosed with complete placenta previa and considered at a low risk for placenta accreta by ultrasonography. Cesarean section and subsequent hysterectomy were required, and histopathological analysis revealed a posterior placenta accreta. Discussion: The authors discuss the association of adenomyomectomy and placenta accreta on subsequent pregnancy and conclude that previous adenomyomectomy may increase the risk of abnormal placentation. Therefore, careful treatment is required during the pregnancies of patients with previous adenomyomectomy.

Key words: Adenomyomectomy; Subsequent pregnancy; Placenta accreta; Placenta previa.
red blood cells and 480 ml of fresh frozen plasma.

Postoperatively, the patient’s blood pressure was 120/70 mmHg, and her pulse rate was 80 beats/minute. A complete blood count indicated a hematocrit level of 37.2% and a hemoglobin level of 12.7 g/dl. The patient had an uncomplicated postoperative course and was discharged 14 days later in good health. The neonate was treated with oxygen at the time of delivery and was breathing room air by the second day of life. He had no complications related to feeding and had gained appropriate weight by the time of his discharge with the mother.

The excised placental specimens are shown in Figure 1B. Macroscopically, there was no evidence of abnormal attachment to the anterior uterine wall; however, there was a clear evidence of abnormal attachment to the posterior uterine wall. Histopathological analysis confirmed the diagnosis of placenta accreta of the posterior uterine wall.

**Discussion**

The influence of adenomyomectomy on subsequent pregnancies is largely unknown. Although a review of the literature identified three case reports of spontaneous uterine rupture after adenomyomectomy [3-5], no reports of severe obstetric complications following adenomyomectomy could be found. A PubMed search using the key words “adenomyomectomy” and “placenta” or “accreta” or “previa” in English yielded no results. To the present authors’ knowledge, this is therefore the first report of a case of placenta previa and placenta accreta following laparoscopic adenomyomectomy.

Most cases of placenta accreta are associated with a history of one or more cesarean sections, while the current case presents an instance of placenta accreta that occurred in a patient with no history of previous cesarean section. A previous study suggested no association of prior myomectomy with higher risks of uterine rupture or placenta accreta [6]. Gyamfi-Bannerman et al. analyzed the subsequent pregnancies of 176 women who underwent myomectomy and identified no cases of uterine rupture or placenta accreta [6]. Because adenomyomectomy does not involve the uterine cavity, obstetricians may not consider it a risk factor for placenta accreta. However, the present authors believe that this report suggests otherwise: that the patient’s previous adenomyomectomy of the posterior uterine wall may have caused subsequent posterior placenta accreta formation during pregnancy. If adenomyomectomy is indeed a contributing factor to the development of placenta accreta, they propose that any patient with previous adenomyomectomy should be considered high risk for placenta accreta, even if she does not develop placenta previa.

Some reports have shown that a predelivery diagnosis of placenta accreta is associated with decreased maternal hemorrhagic morbidity [7, 8]. Therefore, it is important to diagnose placenta accreta accurately before delivery whenever possible. In the present case, ultrasonography suggested a low risk for placenta accreta, but the macroscopic and histopathological examination of the placenta after delivery indicated clear placenta accreta in the posterior wall, with abnormal attachment. Ultrasonography reportedly has a lower degree of accuracy when diagnosing posterior placenta accreta compared with anterior placenta accreta [9]. Therefore, although the ultrasonography findings did not show posterior placenta accreta, it is possible that additional magnetic resonance imaging studies of the posterior uterine wall would have increased the chances of successfully diagnosing placenta accreta before delivery.

The present authors believe that their findings suggest that previous adenomyomectomy of the posterior uterine wall may have caused the subsequent posterior placenta accreta. In this case, they were fortunate that the possibility of placenta accreta had been considered and man-

**Figure 1.** — (A) Ultrasound showing a low risk of placenta accreta of the uterine wall. (B) Macroscopic and histopathological analysis of the placenta indicate placenta accreta of the posterior uterine wall.
aged accordingly because of the diagnosis of placenta previa. However, if adenomyomectomy is indeed a contributing factor to the development of placenta accreta, the authors propose that there is a need for any patient with a previous adenomyomectomy to be considered at high risk for placenta accreta, even if she does not develop placenta previa.

Conclusion

The current authors presented a case of placenta accreta of the posterior uterine wall in a patient who had previously undergone laparoscopic adenomyomectomy. It is possible that the placenta accreta in this case was causally related to the adenomyomectomy and perhaps to the history of adenomyosis itself. More research is needed to confirm this finding. However, it is important that obstetricians are aware of the possible connection between adenomyomectomy and abnormal placentation. Such awareness will help raise the suspicion for placenta accreta in pregnant patients with a history of adenomyomectomy, prompting thorough diagnostic efforts to identify and prepare for the condition before delivery.

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Abscess formation in ovarian endometriomas after failure of mifepristone-induced abortion

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Summary
Objective: To report a case of abscess formation in bilateral ovarian endometriomas after failure of mifepristone-induced abortion. Case Report: A 36-year-old multiparous woman with bilateral ovarian endometriomas conceived spontaneously and received mifepristone to induce an abortion at 35 days’ gestation. Fever and lower abdominal pain occurred 28 days after the abortion. She then underwent surgical curettage for an incomplete abortion complicated by endometritis. Her symptoms and signs became aggravated, and computed tomography showed a large ovarian abscess. She underwent laparoscopic drainage of the abscess plus the enucleation of the ovarian endometriomas, and received intravenous antibiotic treatment. She resumed menstruation one month later and was doing well at the 11-month follow-up. Conclusion: This case demonstrates the importance of combining antibiotic therapy with mifepristone to induce abortions in women with known ovarian endometriomas.

Key words: Endometrioma; Mifepristone; Abortion; Ovarian abscess.

Introduction
Ovarian endometrioma is a benign gynecologic condition that appears as a marker for extensive pelvic endometriosis and is frequently associated with menstrual problems and subfertility [1]. Investigators have reported that nulliparous women, or multiparous women with no more than two children, who experience advanced-stage endometriosis were more apt to develop tubo-ovarian abscess than those without endometriosis [2]. The combination of mifepristone with misoprostol is well adapted to and used extensively in first-trimester medical abortions. In women who have ovarian endometrioma, the use of medical abortion is not contraindicated and remains the first-line pregnancy termination method of choice [3]. The authors report an unusual case of failed medical pregnancy termination in a woman who had had bilateral ovarian endometriomas that became abscessed and required surgical intervention.

Case Report
A 36-year-old woman, gravida 3, para 2, visited the present outpatient clinic requesting medical termination of pregnancy. She had infertility and bilateral ovarian endometriomas for many years, and her previous pregnancies were conceived by artificial insemination. A cesarean section was performed due to a twin pregnancy 1.5 years previously, and pelvic adhesions were observed. At this office visit, her physician identified a pregnancy of about 35 days’ gestation and bilateral adnexal cystic lesions measuring more than five cm in diameter using vaginal ultrasound. A medical abortion was scheduled as requested.

The treatment protocol consisted of mifepristone 600 mg orally, followed by misoprostol 600 µg orally two days later in the physician’s office. The patient declined oral antibiotics because she was breast feeding her child. Vaginal bleeding with expulsion of blood clots was noted after the initiation of the treatment. She had vaginal bleeding off and on, which was monitored by ultrasound for two weeks after taking mifepristone. Vaginal ultrasound showed retained gestational tissue (Figure 1) indicating an incomplete abortion. Misoprostol 400 µg plus doxycycline 100 mg orally twice daily for seven days were administered. No clinical improvement after treatment was observed; therefore, the patient underwent a surgical abortion with vacuum curettage. The products of conception were histologically confirmed. Three days after surgery, she visited the emergency room due to fever of 39°C. A physical examination showed marked diffuse lower abdominal pain and tenderness. Computed tomographic imaging revealed hyperdense material in bilateral cystic masses; an abscess in the left ovary was diagnosed (Figure 2).

Parenteral antibiotics were administered initially and laparoscopy was performed three days later because of persistent symptoms. A left ovarian endometrioma abscess and right solitary endometrioma were found (Figure 3). Drainage of the abscess and enucleation of the bilateral endometriomas were performed followed by cefazolin two grams daily and gentamicin 180 mg daily in divided doses for one week. The pathology report confirmed the abscess, however, cultures of the abscess and blood grew no bacteria. The patient had an unremarkable postoperative course and was discharged on post-operative day 8. Menstruation resumed one month later, and the patient was in good condition at her 11-month follow-up visit.
For first-trimester medical abortions, misoprostol is used extensively in conjunction with mifepristone. While the regimen varies in Taiwan, the most common government approved regimen for medical abortion before 49 days of gestation is 600 mg of oral mifepristone followed by misoprostol two days later in the physician’s office. The US Food and Drug Administration, the International Federation of Obstetricians and Gynaecologists, and the World Health Organization recommend 200 mg of mifepristone followed by misoprostol 400 µg orally two days later [3, 4]. Nonetheless, the overall efficacy rate among different ethnic groups is close to 95%, and the reported complications requiring hospitalization are less than 0.5 % [3–5]. Failures are related to abortion after 49 days of gestation, lower doses of misoprostol, multiple pregnancies, and uterine anomalies [5–7]. The present patient had no related medical condition and received the standard approved regimen.

Regardless of the misoprostol dose, the Planned Parenthood Federation of America changed its medical abortion protocol at the end of March 2006. Vaginal administration of misoprostol was discontinued and replaced by buccal administration to reduce the risk of infection. Routine antibiotic coverage (doxycycline 100 mg orally twice daily for seven days starting on the same day as mifepristone) and ceftriaxone 125 mg intramuscularly in a single dose for gonorrhea, when considered appropriate, were also provided [8]. There was a 93% reduction in the rate of serious infections following the switch to buccal misoprostol and routine antibiotic coverage. Serious infections, including pelvic abscesses, became extremely rare. The leniency handling the denial of using oral antibiotics due to breast feeding in the present patient was a major predisposing factor causing severe infection in the failed abortion.

Endometriosis is a common, benign, estrogen-dependent gynecologic disease that affects 6% to 10% of all women. Recently, investigators reported that it is associated with subclinical pelvic inflammatory status. Abscess formation in endometriomas occurs after surgical procedures such as cyst aspiration, oocyte retrieval, and diagnostic hysteroscopy [2, 9, 10]. Preexisting old blood in endometriomas and the pelvic area could contribute to bacterial growth. In the current case, the patient experienced prolonged vaginal spotting after the failed medical abortion, surgical vacuum curettage, and the presence of bilateral ovarian endometriomas. The development of a subsequent pelvic infection was a reasonable assumption.

Ovarian endometrioma is an asymmetric disease and is found more frequently on the left than the right side of
the body [11]. Retrograde menstruation and the anatomical differences of the left and right hemipelvis are considered the main predisposing factors. The patient developed a left-side abscess. Tubo-ovarian abscess is mainly an ascending infection, and theoretically, just like endometrioma, it is liable to occur on the left side. However, one recent study did not demonstrate a relationship between tubo-ovarian abscess and anatomical location [12].

Gynecologists have long utilized laparoscopy for diagnosing and treating reproductive and gynecological lesions. However, the use of laparoscopy in the management of intra-abdominal abscess is uncertain. The development of new techniques and instruments for laparoscopy has provided clinicians with the possibility of direct visual confirmation of intra-abdominal conditions, allowing the performance aggressive procedures. Abscesses occurring in ovarian endometriomas, similar to abscesses in severe pelvic endometriosis, often have more aggressive and complicated courses than abscesses in the absence of endometriosis. Furthermore, abscesses associated with endometriosis and endometriomas are, usually, refractory to antibiotic treatment [13]. As in the present patient, laparoscopy enabled the clinician to confirm the diagnosis, remove infected tissue in the pelvis, and perform adequate treatment.

In summary, in order to avoid a failed medical abortion followed by a severe pelvic infection, oral administration of mifepristone should be combined with antibiotics for one week. Thereafter, patients with known ovarian endometriomas should be monitored with serial weekly ultrasound examinations. An alternative method would be to consider a surgical abortion. When dealing with a tubo-ovarian abscess and unsuccessful empirical antibiotic therapy, early surgical intervention improves diagnostic precision and helps accurately determine disease severity; optimal management may then follow.

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Iatrogenic parasitic myoma on the peritoneum of the right pelvic wall

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Summary

Uterine myoma, the most common form of uterine tumor, occurs in approximately 25% of reproductive-aged women. Parasitic myoma, which outgrows its uterine blood supply and obtains a secondary blood supply from another organ such as the omentum, is rare. It is extremely rare if it is on the peritoneum of the right pelvic wall. Only a few cases have been found in this location so far. Here, the authors report an interesting case of parasitic myoma on the peritoneum of the right pelvic wall. They conclude with seven key points, which should be paid more attention to avoid iatrogenic parasitic myoma.

Key words: Parasitic myoma; Iatrogenic parasitic myoma.

Introduction

Uterine myoma, which is the most common form of uterine tumor, occurs in approximately 25% of reproductive-aged women [1]. Parasitic myoma is a rare type of pedunculated subserosal myoma, which is partially or completely separated from the uterus and receives alternative blood supply from another source such as the omentum and mesenteric vessels [2, 3]. There are three theories regarding the pathogenesis of parasitic myomas, and the most prevalent one is iatrogenic cause. Most patients initially have non-specific symptoms including pelvic pain, dyspareunia, or abnormal vaginal bleeding. A diagnosis of parasitic myomas is often an incidental event to an operation for concomitant myomas. They can sometimes cause secondary symptoms, which depends on their location [4]. Further examinations such as measurement of CA 125 concentration and MRI are usually performed. The authors report an interesting case of parasitic myoma on the peritoneum of the right pelvic wall. They conclude with seven points below, which should be paid more attention to: 1) systematic survey the entire cavity, 2) complete removal of tissue pieces, 3) maintain awareness of the potential risk, 4) irrigate the abdomen and pelvis, 5) exclusion of malignancy is mandatory, 6) include parasitic myoma in the differential diagnosis and 7) long-term follow-up.

Case Report

A 33-year-old woman, gravida 6, para 1, came to the authors’ clinic in August 2013, complaining of pain in the abdomen for two days. She had undergone a laparoscopic myomectomy at another medical institution in 2007. She did not use contraception regularly. She used emergency contraceptive pill (levonorgestrel 0.75 mg) six to seven times per year. Clinical examination revealed a bulky uterus with a mass in the pouch of Douglas but separate from uterus. Ultrasound examination revealed a hypoechoic solid mass of 7.3×6.0×4.2 cm in the Pouch of Douglas but separate from uterus (Figures 1A, 1B). MRI: a lesion in the Pouch of Douglas. Low signal intensity on T2WI images (Figures 2A, 2E), intermediate signal intensity on T1WI images (Figure 2B), high signal intensity on DWI images (Figure 2C), and homogeneous enhancement (Figure 2D) were found. The signal intensity of the lesion in enhancement phase was lower than that of uterus muscle. Concentrations of CA125, AFP, CEA, and CA199 were normal. Papanicolaou smear was normal. The patient was adequately informed of the possible risks and benefits of laparoscopic surgery and signed a written consent agreeing to undergo laparoscopic exploration. The case was approved by the Institutional Review Board of the Peking University First Hospital. Laparoscopic myomectomy was performed. In addition, a large parasitic myoma (approximately seven cm in diameter) was found attached to the posterior peritoneum (Figures 3A, 3B), and the pedicle division seemed to be on the posterior uterine wall (Figure 4. Myomectomy was performed – first coagulating and then cutting the pedicle of the myoma. A small second parasitic myoma (approximately three cm in diameter) was found attached to the left mesosalpinx (Figure 5), which was also removed laparoscopically. The pelvic mass weighed 140 grams. The tumor was removed using a morcellator. Pathological diagnosis was leiomyoma with hyaline de-
Figure 1. — A mass of 7.3 cm (A) × 6.0 cm (B) × 4.2 cm (A) in the Pouch of Douglas but separate from uterus there is a hypoechoic solid tumor.

Figure 2. — A lesion in the Pouch of Douglas. Low signal intensity on T2WI images (A, E), intermediate signal intensity on T1WI images (B), high signal intensity on DWI images (C), and homogeneous enhancement (D) were found. The signal intensity of the lesion in enhancement phase was lower than that of uterus muscle.

Figure 3. — (A) A large parasitic myoma (approximately 7 cm in diameter) was found attached to posterior peritoneum. (B) After cutting the pedicle of the myoma, The arrow shows the pedicle attached to posterior peritoneum.
Iatrogenic parasitic myoma on the peritoneum of the right pelvic wall

Figure 4. — The arrow shows the pedicle division, which seems to be on the posterior uterine wall.

Figure 5. — A small second parasitic myoma (approximately three cm in diameter) is found attached to the left mesosalpinx.

Figure 6. — (A) Intersecting fascicles of cytologically bland, spindled cells with cigar-shaped nuclei and eosinophilic cytoplasm (HE ×20). (B) An area with hyaline degeneration (HE ×20). The spindled cells of leiomyoma express ER (C) and PR (D) diffusely.
Parasitic myoma is a rare type of pedunculated subserosal myoma, which is partially or completely separated from the uterus and receives alternative blood supply from another source such as the omentum and mesenteric vessels [2, 3]. Although the exact mechanisms for the subsequent growth of parasitic myoma are not well understood [5], it seems reasonable to hypothesize that exposure of retained myoma fragments to sex steroid hormones and/or growth factors may result in the growth of parasitic myoma later [5]. Parasitic myomas were found most frequently parasitizing to the omentum, and second most frequently parasitizing to the sigmoid colon [4, 6-14]. However, the present authors report an interesting case of parasitic myoma on the peritoneum of the right pelvic wall, with positive estrogen and progesterone receptors (ER 90%++, PR90%+++). Moreover, she used emergency contraceptive pill six to seven times per year.

Parasitic myoma, which seems to be a rare disorder developing from the natural history of pedunculated myomas, has become increasingly reported over the last decade [4, 6-14]. Parasitic myomas were described for the first time by Kelly and Cullen [15]. There are three theories regarding the pathogenesis of parasitic myomas. The first hypothesis is that parasitic myoma spontaneously develops from pedunculated myoma, and it needs more blood supply to grow and to maintain itself, so that it adheres to its surrounding structures and separates itself from the uterus. The second hypothesis is that parasitic myoma is associated with previous uterine surgery. During the morcellation of myomas, small particles of myoma may be left in the pelvic, which may implant in normal tissues [4]. The third hypothesis is that parasitic myoma is associated with restriction of blood supply to the uterus. Therapeutic administration of gonadotropin-releasing hormone agonists restricting blood supply is effective in treating the symptoms of a myomatous uterus. Similarly, other minimally invasive procedures such as uterine artery embolization and MR(f)US are also used to manage symptomatic myomas. Some researchers suggested that parasitic myomas developed after these two kinds of treatments [14, 16]. Parasitic myoma is considered iatrogenic disease in the last two hypotheses.

Most patients have non-specific initial symptoms including pelvic pain, dyspareunia or abnormal vaginal bleeding. Diagnosis of parasitic myomas is often an incidental event to an operation for concomitant myomas, such as the present second parasitic myoma attached to the left mesosalpinx. They can sometimes cause secondary symptoms, which depends on their location [4]. Parasitic myomas occur at various sites, including the port site [7], intestines, peritoneum, and omentum in the abdominal cavity. A history of hysterectomy, myomectomy or presence of concurrent uterine myomas may support the diagnosis in many cases; however, usually the finding of a heterogeneous pelvic mass at transvaginal ultrasonography warrants exclusion of malignancy, and further examinations such measurement of CA125 concentration and MRI are usually performed. Due to its multiplanar detection capability, MRI is the most reliable technique in such cases. It can accurately demonstrate the location of the tumor relative to adjacent structures such as the ureter, bladder, or rectum, which is critical for surgical planning. Typical leiomyomas demonstrate low to intermediate signal intensity on T1-weighted images and low signal intensity on T2-weighted images. Myxoid degeneration and necrosis may be visible as high signal intensity areas on T2-weighted images. Another common variant form seen on both T1- and T2-weighted images is a cobblestone-like appearance caused by hyaline degeneration, with high-signal intensity foci representing areas of infarction caused by rapid growth [17]. The present patient’s MRI showed low signal intensity on T2WI images, intermediate signal intensity on T1WI images, high signal intensity on DWI images, and homogeneous enhancement. Serum CA125 in patients with parasitic myoma is normal or slightly increased. The present patient’s serum CA125 was normal. Ghamande et al. [18] reported a patient with parasitic myoma presenting a CA125 level of 1,539 U/ml. The patient underwent an exploratory laparotomy and resection of a parasitic fibroid following which the CA125 levels decreased and normalized within a month. Ascites are rare symptoms of parasite myoma. Kebapci et al. [19] reported a patient with parasitic myoma presenting massive ascites. Ascite and pleural effusion disappeared six months after surgery.

In the last ten years, there has been an increase in the reports of parasitic myomas in the literature, and they are found to be largely associated with previous surgeries. The increasing report of incidence of iatrogenic parasitic myomas in the literature might be caused by increased use of minimally invasive surgery. Advances in laparoscopic techniques and instrumentations have enabled physicians to avert laparotomy in many cases, resulting in practical benefits over conventional surgeries. Since the 1990s, the laparoscopic approach has gained popularity, and currently, total hysterectomy [20], subtotal hysterectomy [21], and myomectomy [22] are frequently performed by laparoscopy in many medical institutions around the world. Laparoscopic myomectomy is usually a feasible option, irrespective of size, site or number of the myomas [23]. However, some long-term complications after morcellation of the uterus have recently been described in the literature [4, 6-14]. There is even a report of a uterine leiomyoma particle growing in an abdominal wall incision [24], and another reported case of parasitic myoma under the dome of the diaphragm [25]. Tissue morcellation, especially in large myomas, may be very time-consuming, therefore, tissue pieces may spread in the abdominal cavity. Morcellation enables removal of large specimens at laparoscopy, however, even using a morcellation bag [26], there is still risk for incomplete removal. According to a recent report, retained
fragments occur in 0.57% of subtotal hysterectomies [11]. This is especially true in obese patients in whom the visceral fat makes inspection of the peritoneal cavity more difficult. These retained fragments usually become infarcted and cause abdominal pain, which necessitates immediate removal of the mass. There have been reports of retained tissue becoming necrotic and causing severe peritonitis [27]. In the present case, the patient had a history of myomectomy. In terms of the iatrogenic pathogenesis of parasitic myomas, it is considered that fragments from incomplete removal of a myoma during the previous operation could have implanted in the posterior peritoneum and formed the stalk.

The present authors conclude the following seven key points below, which should be paid more attention to: First of all, systematic surveying of the entire cavity with meticulous surgical technique is necessary. Second, all tissue pieces that are morcellated should be completely removed. Even small bits displaced into the upper abdomen can result in parasitic fibroids [25]. Third, it is important for us as physicians and surgeons to maintain awareness of this potential risk to the patients. Fourth, placing the patient in reverse Trendelenburg position after morcellation and repeatedly irrigating the abdomen and pelvis with normal saline may be helpful to wash out small pieces in pelvis [6]. After intraperitoneal lavage, a thorough examination of the abdomen and pelvis should be performed and all the remaining tissues should be removed. Fifth, if morcellation is anticipated or required, exclusion of malignancy is mandatory. In all patients who are at risk of uterine malignancy (e.g., postmenopausal or with intermenstrual bleeding) and will undergo the surgical procedure, uterine biopsy and cervical cytologic analysis should be performed before surgery to reduce risk of possible spread after morcellation of a malignant tumor, despite the possibilities of false-negative result or other potential causes leading to the patient’s symptoms. Sixth, in patients who have pelvic masses with a history of morcellation, iatrogenic parasitic myomas should be considered in the differential diagnosis [28]. Seventh, it is necessary to perform a long-term follow-up.

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Repeated cesarean scar pregnancy – Case report

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Summary
Cesarean scar pregnancy (CSP) is a rare location of an ectopic pregnancy implanted within a scar from previous cesarean section, separated from the endometrial cavity. The prevalence ranges from 1:1,800 to 1:2,226 pregnancies. It is a potential life-threatening condition, and if misdiagnosed, can cause serious maternal morbidity from uterine rupture with massive hemorrhage and even death. Until now, no universal treatment guidelines have been established, with treatment options ranging from systemic or local injection of methotrexate (MTX), suction curettage under ultrasound control to surgical treatment, including hysterectomy and wedge resection of the ectopic pregnancy, via laparotomy or laparoscopy. The authors present a case of a 42-year old woman with two consecutive CSPs. First CSP was unsuccessfully treated conservatively, followed by ultrasound guided vacuum aspiration of the uterine cavity. Second CSP was treated by laparotomy and a wedge excision of a CSP and repair of a scar with interrupted sutures. The authors also discuss diagnostic pitfalls and treatment modalities.

Key words: Ectopic pregnancy; Uterine scar pregnancy; Cesarean section.

Introduction
Cesarean scar pregnancy (CSP) is a rare location of an ectopic pregnancy implanted within a scar from previous cesarean section, separated from the endometrial cavity. The first report was dated in 1978 [1] and reports are increasing exponentially ever since. The prevalence is ranging from 1:1,800 [2] to 1:2,226 [3] pregnancies, estimated on relatively small samples from single centers. With cesarean section rates rising worldwide, one can assume that this entity will become more common. It is a potential life-threatening condition, and if misdiagnosed, can cause serious maternal morbidity from uterine rupture with massive hemorrhage and even death. Until now, no universal treatment guidelines have been established.

The authors report a case of a patient with two consecutive CSP which is by their knowledge unreported in the literature. They also report different treatment regimens, both with good short-term outcomes, however, without later successful ongoing pregnancy.

Case Report
The authors present a case of a 42-year old woman (gravida 6, para 2), with history of two elective isthmic transverse cesarean sections and four missed abortions. The first cesarean delivery was performed due to breech presentation, and second due to previous one. She presented in six weeks of pregnancy with vaginal spotting. Her serum β chorionic gonadotropin (βHCG) level was 15,812 and at follow-up increased to 17,475 IU/L. Transvaginal ultrasound showed an embryonic pregnancy located within the lower uterine segment in a scar of previous cesarean section. An expectant management was initiated, however, the level of βHCG was slowly rising. Therefore, the pregnancy was terminated via vacuum aspiration of uterine cavity guided by transabdominal ultrasound. Her βHCG levels were monitored until they returned to a normal level 14 days postoperatively. Five months later she referred in eight weeks of pregnancy. Transvaginal ultrasound showed a gestational sac within a scar of a previous cesarean section with positive fetal heart-beat (crown-rump length 11 mm). Magnetic resonance showed round lesion with high signal in T2-weighted image measuring 14×9 mm in a projection of uterine scar (Figure 1). Two days later, transvaginal ultrasound showed an absence of an embryonic vitality. Due to recurrence of ectopic pregnancy in a cesarean scar, the authors decided to offer a patient a surgical correction of a uterine scar. Laparotomy was performed, with wedge excision of a CSP and repair of a scar with interrupted sutures. Her βHCG levels were monitored until they returned to a normal level 18 days postoperatively. Two years later she referred again in six weeks of pregnancy with vital intrauterine pregnancy (crown-rump length five mm). One week later she referred with heavy vaginal bleeding. Transvaginal ultrasound showed complete abortion.

Discussion
CSP is a rare location of an ectopic pregnancy implanted within a scar from previous cesarean section, separated from the endometrial cavity. The exact cause and mechanism of CSPs are not well understood. The most probable mechanism that could explain scar implantation is invasion of the myometrium through a microtubular tract between the cesarean section scar and the endometrial canal [4]. The incidence of CSP seems to be increasing lately, possibly because of the increased use of cesarean section and more wide-spread use of transvagi-
nal ultrasound scan as a diagnostic method. Wang et al. [5] proposed useful diagnostic criteria for CSP by using transvaginal ultrasonography. It is still uncertain whether the risk of CSP is related to the number of prior cesarean sections. Some authors have reported that between 50% and 72% of CSPs occur after two or more prior cesarean sections [6]. CSP is a condition with a possible long term morbidity [7] and even a life-threatening event. There are still doubts about treatment options, ranging from expectant management [8] to surgical approach [9]. The prognosis for uneventful term pregnancy is very poor, with only anecdotal reports regarding vital term CSP with favorable fetal outcome [10]. Therefore, the current policy is to recommend termination once the proper diagnosis is made.

The non-surgical strategy seems to be the most appropriate option when the trophoblast reaches near the bladder wall. Furthermore, the proper candidates for medical management should be hemodynamically stable and pain free, with unruptured CSP less than eight weeks and myometrial thickness less than 2 mm between the gestational sac and bladder. The medical regimens include systemic methotrexate (MTX), local embrocides (MTX, potassium chloride or hyperosmolar glucose) or combined systemic methotrexate (MTX, local embryocides (MTX, potassium chloride or hyperosmolar glucose) or combined management should be hemodynamically stable and pain free, with unruptured CSP less than eight weeks and myometrial thickness more than 3.5 mm [11,12]. Other authors report high complication rate after this procedure, from intraoperative hemorrhage to high failure rate. Hysteroscopy is a method which allows direct visualization of gestational sac along with the vessels at the implantation site, allowing their coagulation. Other benefits include short follow-up, avoidance of toxicity of MTX, and shorter period for achieving pregnancy. The potential concern is the possibility of a bladder injury, reported after hysteroscopic approach. Deans and Abbott described a macrohematuria followed by hysteroscopic removal of CSP, resulting in a macrohematuria in one patient. However, the condition was self-limited, with no need for further treatment [13]. A promising advance is a use of laparoscopy guided hysteroscopy, especially in cases with deeper implanted CSP [14]. Laparotomy followed by wedge excision of CSP is a conventional surgery for cases with uterine rupture and has an advantage of complete removal of CSP along with simultaneous reconstruction of a scar. Vial et al. [15] suggested that surgical resection of the old scar and new closure should be offered even if recurrence is unlikely. Successful surgical resection of the old scar and new closure without severe side effects have been reported.

Conclusions

The main goal in the management of CSP is the preservation of the uterus for a future fertility. However, until now, there are still no universal management guidelines.

References


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Introduction

Placental mesenchymal dysplasia (PMD) is a rare placental disease with unknown cause. The pathogenesis of PMD remains unclear. It was first reported by Jauniaux et al. [1] in 1997. Only approximately 100 cases of this disease have been reported so far in English literature. Zeng et al. [2] conducted an investigation at the Department of Gynecology and Obstetrics, McGill University of Quebec, Canada for 18 years (1991–2009). A total of 95,000 babies were delivered; however, only two cases were diagnosed with placental mesenchymal dysplasia. Thus, the incidence rate was only 0.02‰.

The sonographic and macroscopic features of PMD are similar to partial hydatidiform mole. It is difficult to distinguish these two diseases during prenatal care. Because of the malformation of the placenta, the incidences of the obstetric complications and the poor perinatal outcomes are higher than usual. Here, the authors report a case of PMD with a good outcome.

Case Report

In this case, the gravida was 23-years-old. She usually had regular menstruation. This was her first pregnancy. She did not experience nausea, vomiting, and other reactions in early pregnancy. She also had no history of vaginal bleeding, drainage, toxic substance and drug use, and radiation exposure. At 11+6 weeks, inspection card was used for regular prenatal examinations. At 13+6 weeks, the crown-rump length and NT of the fetus in the uterus were 7.46 and 1.3 mm, respectively. A honeycomb clump (8.8×4.2×7.6 cm) was attached to the uterine back wall, revealing an abnormal intrauterine echo group (suspected partial malignant mole) (Figure 1A). Down’s screening in the middle stage of pregnancy (16+1 weeks) showed that the free β-hCG was 29.07 ng/ml, which indicated a normal result, and APF (MOM) was 6.22 higher than normal. The finding revealed a high risk of open neural tube defect. Targeted ultrasonic examination showed no abnormality. The repeated B-ultrasonic examinations during pregnancy showed abnormal intrauterine echo group. At 30+3 weeks, intrauterine growth restriction was found. Ultrasonic examination revealed that biparietal diameter, femur length, head circumference, and abnormal circumference of the infant were 7.0, 5.1, 25.9, and 22.6 cm, respectively. The patient was only asked to improve nutrition without special drug treatment. No abnormalities were revealed in the remaining pregnancy inspection. An increase in gravida body weight of approximately 10 kg was found.

At 35+5 gestational weeks, premature rupture of membranes occurred and the patient was immediately admitted to the hospital. After admission, the patient gave birth to a live premature female infant. The premature baby weighed 1,800 grams and measured 43 cm in length without obvious deformity. The Apgar score of the newborn was 10-10-10. The placenta measured approximately 23×20×4 cm and weighed 760 grams. Intact placenta and membranes were observed. Approximately 0.2–1 cm beaded blisters were directly found distributed in the maternal placenta. Approximately 8×7 cm area of the beaded structure was found clustered in the maternal placenta (Figure 1B). No abnormalities in the navel cord, which adhered to the placenta paracentralis, were found.

On the second postpartum day, blood examination showed ThCG 4335.0 mIU/ml. The blood ThCG on the 18th postpartum day was reduced to 7.5 mIU/ml, which was within the normal range.

On the first day after birth, the baby was admitted in the Neonatology Department because of pathological jaundice and treated for five days. No abnormalities were found in the appearance, phenotype, and all blood examination indicators of the newborn. Since the birth of the baby one year later, no abnormalities were also observed in her growth and development.

Postpartum placental pathology showed less mature single placenta, placental mesenchymal dysplasia (Figure 1C), and placental focal infarction accompanied by calcification, mild chorioam-
Discussion

PMD can easily be misdiagnosed as partial mole on imaging because the disease is difficult to distinguish. This disease is mostly diagnosed through postnatal pathological examination. Its main pathological features are cystic expansion manifested in the placental stem, but no trophoblastic hyperplasia. The hCG of the patient without trophoblastic hyperplasia is similar to that in normal pregnancies. The hCG in this case was sharply reduced after operation and then restored to normal on the 18th postpartum day.

Most of the patients diagnosed with PMD have normal karyotype [3, 4]. However, some studies report that PMD may be related to abnormal karyotype, such as androgenetic-biparental mosaicism [5] and cytogenetic ploidy [6]. The incidence of the disease is also found more in females than males [6]. The newborn described in this paper was female. Unfortunately, chromosome testing was not conducted because of the disagreement from the patient and her family.

According to a current literature, placental echo abnormality is found in most PMD patients at the middle stage of pregnancy or 13–20 weeks, showing honeycomb performance without fetal malformation [7]. During this time, the disease can be distinguished from partial mole through blood hCG inspection, amniocentesis, or umbilical cord puncture [3]. Doctors always inform patients of abnormal findings during prenatal check-ups. Some pregnant women will opt to terminate the pregnancy [4], whereas some will choose to continue the pregnancy. However, other pregnant women do not undergo relative inspection to continue the pregnancy because of the lack of knowledge on this disease. The patients who continue the pregnancy are more at risk of suffering from obstetrical complications, such as severe preeclampsia [8], oligohydramnios [8], intrauterine fetal growth restriction [3], premature birth [9], hemangioma [9], and in severe cases, fetal death [6, 8]. Pregnant patients rarely continue the pregnancy until the late stage and have uncomplicated delivery of live newborn babies [9]. In this paper, placental echo abnormality was initially found at 13+6 weeks of pregnancy. The patient had a normal hCG. No fetal chro-
mosomal abnormalities were found and no abnormalities were also observed in the remaining inspections except for fetus growth restriction. When pregnancy reached 35+5 weeks, her weight increased approximately ten kg, meeting the weight increase standard. However, the fetus growth was restricted, indicating that PMD may affect maternal–fetal nutrient exchange.

Approximately 20%–50% of placental mesenchymal dysplasia in newborns is accompanied with fetal Beck-with–Wiedemann syndrome [4, 6], which is a congenital overgrowth. Infants may develop this disorder before birth [10]. After birth, newborns may have umbilical hernia, macroglossia, visceral mast, adrenocortical cell hypertrophy, hypoglycemia, and other diseases. The case in this paper did not have the aforementioned abnormalities after birth. During one year follow-up, no abnormalities in growth and development were found. The authors inferred that the influence of PMD to the fetus only existed in the uterus. When the baby was born, there was no influence on the growth of the normal phenotype baby.

Conclusion

PMD is a rare malformation of placenta. It is difficult to distinguish from the partial mole during prenatal care and diagnose definitely. If the prenatal examination indicates an abnormal placental echo, doctor should be aware of this disease and perform related examinations, such as karyotype and hCG. More frequent prenatal care is needed to find the obstetric complications, treat the complications in a timely manner, and improve the outcome of pregnancy.

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References


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