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Current diagnosis and management of ovarian cysts

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Summary

The epidemiology of ovarian cysts is unclear due to the lack of consistent reporting and a high likelihood of spontaneous resolution. In the USA, postmenopausal women have an ovarian cyst incidence of 18% over a 15-year period. Worldwide, about 7% of women have an ovarian cyst at some point in their lives. In Europe, a large screening trial revealed a 21.2% incidence of ovarian cysts among healthy postmenopausal women. The American College of Obstetricians and Gynecologists (ACOG) stated that simple cysts found on ultrasound may be safely followed without intervention, even in postmenopausal women. These cysts are not likely cancer precursors, nor markers of increased risk, and can be managed conservatively. Simple ovarian cysts appear to be stable or resolve by the next annual examination. These findings support recent recommendations to follow unilocular simple cysts in postmenopausal women without intervention. For those patients, ovarian cancer screening and follow up include a CA-125 blood test and transvaginal ultrasonography (TVU) at baseline, an annual TVU for up to three additional years, and annual CA-125 tests for up to five years beyond baseline. The TVU screening examination is considered positive (abnormal and suspicious for ovarian cancer) when findings included: 1) ovarian volume greater than 10 cubic cm; 2) cyst volume greater than ten cubic cm; 3) any solid area or papillary projection extending into the cavity of a cystic ovarian tumor of any size; or, 4) any mixed (solid/cystic) component within a cystic ovarian tumor. Women with positive screening examinations are referred to gynecologic oncology unit for follow-up investigation. Diagnostic consideration and surgical management of ovarian cysts are discussed.

Key words: Ovarian cysts; Diagnosis; Surgical management; SPA robotic surgery for ovarian cysts; Large ovarian cysts.

Introduction

In clinical practice, ovarian neoplasms are a common problem affecting pre- and postmenopausal patients. They are the fourth most common reason for gynecologic hospital admission in the United States. It has been estimated that approximately 10% of women in the United States will undergo surgical procedure for a suspected ovarian neoplasm during their lifetime [1]. Functional ovarian cysts and benign neoplasms make up most of these abnormalities.

Most functional cysts resolve and can be observed, although they can cause menstrual irregularities, pain, and rare intraperitoneal bleeding. There are several histopathologic types. Ovarian cystadenomas are benign tumors with simple cyst walls, small in size, and more likely to be unilateral. Mucinous cystadenomas are multicystic and could achieve large size. Mature cystic teratomas are the most frequent germ cell tumor and are composed of one or more of the three primitive germ cell layers. They vary in size and presentation. They are often asymptomatic and may be more prone to torsion.

Ovarian thecomas originate in the medulla. They produce estrogen, and may have concomitant endometrial hyperplasia or neoplasia. Ovarian fibromas are likely to originate in the ovarian cortex, are usually asymptomatic, can grow to a large size, and may result in Meigs syndrome with ascites and pleural effusions.

Endometriomas are a result from the invagination of endometriotic tissue into the ovary. The ideal treatment of this tumor is cystectomy. The American College of Obstetrics and Gynecology (ACOG) and the Society of Gynecologic Oncologists (SGO) published joint guidelines for referral to a gynecologic oncologist. According to these guidelines, the provider should refer premenopausal women who have a pelvic mass that is suspicious for malignant ovarian neoplasm based on a CA-125 blood test and transvaginal ultrasonography (TVU) at baseline, an annual TVU for up to three additional years, and annual CA-125 tests for up to five years beyond baseline. The TVU screening examination is considered positive (abnormal and suspicious for ovarian cancer) when findings included: 1) ovarian volume greater than 10 cubic cm; 2) cyst volume greater than ten cubic cm; 3) any solid area or papillary projection extending into the cavity of a cystic ovarian tumor of any size; or, 4) any mixed (solid/cystic) component within a cystic ovarian tumor. Women with positive screening examinations are referred to gynecologic oncology unit for follow-up investigation. Diagnostic consideration and surgical management of ovarian cysts are discussed.

Diagnostic consideration

Sonography (particularly three-dimensional sonography), magnetic resonance imaging (MRI), and computed tomography (CT) imaging are each recommended for differentiating malignant from benign ovarian masses. Serum CA-125, as a standalone modality is not diagnostic for ovarian malignancy.

Surgical procedures

1) Laparoscopy is a reasonable alternative to laparotomy. The choice between laparoscopy and laparotomy should be based on patient and clinician preferences. The benefits of laparoscopy include reduced postoperative analgesic re-
quirement, earlier mobilization reducing chances of deep venous thrombosis (DVT), cosmetic advantages, earlier discharge from the hospital, and return to normal activity. One should note that fertility-preserving surgery is an acceptable alternative to more extensive surgery in patients with low-malignant-potential tumors, those with well-differentiated surgical Stage I ovarian cancer, and who have the desire to conceive in the future. Full discussion, regarding this option with a gynecologic oncologist is important. It is estimated that approximately 80% of benign ovarian tumors can be successfully removed using minimally invasive technique. The additional advantages of this approach include: improved magnification and avoidance of unnecessary laparotomy in patients with benign ovarian tumors [3]. It has been shown that laparoscopic ovarian cystectomy is associated with decreased postoperative adhesion formation compared with laparotomy [4]. Cases of dermoid cysts and endometrioma spillage should be avoided as they could cause chemical peritonitis and increase the risk of postoperative adhesions. Rupture of dermoid cyst, during surgery, can cause granulomatous reaction [5].

It was noted that ovarian cystectomy/oophorectomy performed by laparoscopy was associated with less postoperative pain than laparotomy [6-7]. It has been shown that the incidence of operative complications such as transfusion rate, visceral damage, infection, thromboembolism, and perioperative mortality was similar between the laparoscopy and the laparotomy group. However, the duration of surgery tended to be longer in the laparoscopy group than in the group who had laparotomy [6]. There was no difference in the recurrence rate of ovarian tumors between the two study groups. All these pooled results were homogenous. In the study by Damiani et al. [8], laparoscopy was found to have a lower surgical cost than laparotomy [mean difference in cost ($1,000 USD in 1993)].

There are some disadvantages for utilizing laparoscopy in treating ovarian cysts, for example left-side adnexal masses in patients who have undergone hysterectomy can be difficult, because resection on and around the rectosigmoid and its mesentery is frequently required. Nonetheless, the feasibility often can be determined only after laparoscopic inspection. Maiman et al. [9] reported on laparoscopic mismanagement of ovarian tumors. The mismanagement included aspiration of malignant cysts without removal (38%), partial removal of malignant cysts (33%), absence of utilization of frozen section (60%), and no serum tumor markers (88%). Delayed laparotomy as a second procedure was noted in 71% of patients, with an average delay of 4.8 weeks between procedures. Rupture of ovarian cyst capsule is another disadvantage of laparoscopy in the oncologic setting. Laparoscopy is more likely than laparotomy to result in capsular rupture, because with laparoscopy masses often must be drained before removal. Webb et al. [10] reported that the five-year survival for the 53 patients with ruptured cysts was 78% compared with 56% for those with unruptured cysts. This retrospective, univariate analysis did not stratify for tumor adherence or high-grade lesions, both of which were more common in the patients with ruptured cysts. The avoidance of ovarian tumor mismanagement is important when suspicious adnexal masses are diagnosed. Certain guidelines must be observed. Inspection of the entire intraperitoneal cavity should be performed first, with special attention paid to the diaphragms, the omentum, and the pelvic peritoneum. Intraperitoneal washings for cytologic testing should be performed before the initiation of any operative procedure. Avoidance of capsular rupture should not be overlooked. The use of large laparoscopic sacs, drainage of cysts or morcellation of masses may be accomplished within these sacs, allowing removal through small abdominal wall or colpotomy incisions without peritoneal contamination. If intra-abdominal cystic drainage is necessary for very large masses. Also, the capsular puncture site should be closed after drainage. The use of frozen section is critical to avoid delay in definitive surgical management and chemotherapy. In addition, the patient should be physically and psychologically prepared for cancer surgery. Some surgeons see some disadvantages of colpotomy, including incisional infection, peritonitis, and technical complexity, particularly in patients after hysterectomy; however bringing the opening of the collection bag out an anterior abdominal wall incision, is likely to have comparable results.

2) Single port access (SPA) robot-assisted laparoscopic surgery utilizing da Vinci surgical system may be used as a minimally access invasive surgery in cases of ovarian cysts. Robotic surgery is feasible and safe for patients with either benign or malignant gynecologic disease even with severe pelvic adhesions. The ease of operating the robotic system may overcome the limitations and long learning curve of conventional laparoscopic surgery in complicated conditions. The success of robotic surgery depends on teamwork. There have been reports showing that a gynecologist can master robotic surgical staging in 20 patients [11]. My own experience is that, there is no significant difference between novice and expert laparoscopists when learning to master an operation using the da Vinci surgical system. There are some disadvantages for this surgical system, namely: high cost, bulkiness of the device, loss of haptic feedback, and inconvenience for the assistant to manipulate the uterus and to exchange instruments. This could be improved as the robotic and surgical instrumentation technologies evolve. It is worth noting that in case of discovery of a malignant mass, the robot does not allow access to the upper quadrants of the abdominal cavity and requires de-installation of the robot and 180° rotation of the operating table before placement of new trocars to complete the procedure [12,13].

In cases of malignant ovarian tumor, robot-assisted laparoscopic surgery for those patients is safe and effective.
alternative to laparoscopic and laparotomy surgery. It has the advantage of three-dimensional vision, ergonomic, intuitive control, and wristed instrument that approximate the motion of the human hand. It can decrease the incidence of intraoperative complications and postoperative wound complications without significantly increasing operative time or blood loss. The procedure is cost-effective with acceptable operative, pathological, and short and long term clinical outcome. It retains the advantage of minimally invasive surgery [14]. The disadvantages of robot-assisted surgery include the cost, bulkiness, and availability of the robot in different hospitals. With the cost of the equipment being as high as two million US dollars, the annual maintenance fees, and the cost of semi-disposal instruments. Additional costs include the extra operating room time needed to assemble, disassemble, and prepare for the robotic portion of the surgery. In addition, it is awkward for the assistant to work around the robot to interchange equipment, manipulate the uterus, and exchange instruments in the accessory ports. In a standard laparoscopic surgery, it is easier and faster to exchange instruments.

Surgical approach to large ovarian cysts

Laparoscopic or robot-assisted laparoscopic management of these cysts could be safely performed following a thorough preoperative assessment of their size and nature in order to achieve complete removal of the ovarian pathology and avoid spillage. In my experience, preoperative Doppler ultrasound, CT, and levels of serum tumor markers are imperative during the preoperative evaluation of patients. Ovarian cysts were considered suspicious in the presence of at least one of the following features: thick/irregular cyst wall, thick septa, and solid papillary projections [15]. The risk of malignancy of ovarian cysts after careful preoperative assessment could be reduced to 0.2% to 0.6%. [16]. Cyst rupture represents common events during surgical management of ovarian cystic masses [17]. The incidence of tumor spillage in laparoscopically managed large ovarian masses varies between 22% and 100%, [16, 18-20], whereas the risk of rupture during laparotomy has been reported to be in the range of 10% to 26% [21]. There are several techniques to prevent spillage during laparoscopy: 1) the use of grasping forceps through the five-mm port site to obliterate the puncture site and minimize spillage [22], 2) the removal of the specimen through a laparoscopic bag [23]. In addition, a thorough peritoneal lavage is recommended at the end of the procedure, especially if spillage has occurred. In my experience, laparoscopic or robot-assisted laparoscopic management of ovarian tumors is feasible for large ovarian cysts and offer additional benefits: decreased hospitalization reduced postoperative pain, faster return to normal activities, and better cosmetic results. There is always a concern about the adverse impact of cyst rupture in cases of malignancy.

The impact of intraoperative cyst rupture in early-stage ovarian cancer is controversial. There is no significant difference in survival or disease-free interval described between patients with iatrogenic Stage IC epithelial ovarian cancer and Stage IA and IB disease. Dembo et al., [23] demonstrated that tumor grade and presence of dense adhesions or ascites are the sole prognostic factors for tumor relapse. Another study showed that the survival rate reached 78% in patients with intact tumor, 87% in those with punctured cysts, and 84% in those with spontaneous rupture [24]. In addition, it has been shown that the degree of differentiation was the most powerful prognostic factor for disease-free survival, followed by rupture before surgery, rupture during surgery, FIGO stage, and age. Histological type, dense adhesions, extracapsular growth, ascites, and size of the tumor had no prognostic value for disease-free survival [25]. Another potential risk is the accuracy of frozen section diagnosis. It is known that specificity and sensitivity are high in ovarian tumors regardless of size [26]. In my view, definite surgery for ovarian tumor, in women of reproductive age should not be performed based on frozen section results prior to the final histopathological report.

Conclusion

The laparoscopic approach to benign ovarian masses offers significant advantages over conventional laparotomy, as it reduces morbidity, hospital stay, and recovery, without increasing the risk of spillage of the cyst contents. In addition, robot-assisted laparoscopic surgery has the advantages of the wrist motion which allows for precise surgery, and suturing than conventional “straight stick” laparoscopy. The three-dimensional vision in robot-assisted surgery provides substantial depth of field perception. Overall, minimally invasive surgery should replace laparotomy in the management of ovarian masses.

References


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Platelet-activating factor acetylhydrolase and premature ovarian failure

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Summary
Premature ovarian failure (POF) appears to be a complex disease entity with several underlying etiopathogenic contributions including the possibility of multiple distinctly different autoimmune mechanisms, in which inflammatory autoimmunity targeted to ovarian-specific germline antigens (e.g., zona pellucida proteins or Mater) or differentiation/regulatory factors (e.g. inhibin-alpha) were regarded as one of the most crucial factors. Platelet-activating factor (PAF) and PAF class oxidized phospholipids stimulate the occurrence and development of inflammation and atherosclerosis. PAF acetylhydrolase (PAF-AH) can hydrolyze PAF and PAF class oxidized phospholipids and eventually prevent the body from the damage of these inflammatory mediators. These findings indicate a potential relationship between PAF-AH and POF thus have major implications for the future health of women who suffer with premature ovarian failure.

Key words: Premature ovarian failure (POF); Platelet-activating factor (PAF), PAF acetylhydrolase (PAF-AH).

Introduction
Premature ovarian failure (POF), generally described as irreversible cessation of menses before the age of 40, has a torturing influence on both women's physical and mental health. It is characterized by hyper-gonadotrophic and hypo-estrogenic amenorrhea and regarded as the final step in the process of aggravation of ovarian dysfunction. Previous population-based studies estimated that about 0.3% ~ 1% adult women experienced POF. The total incidence varied from 1% to 3% due to different ethnicity. Almost 20% ~ 25% patients of primary amenorrhea and 10% ~ 20% of secondary amenorrhea eventually diagnosed as having POF.

Many factors are considered to be correlated with POF. Besides smoking, pelvic radiotherapy, pelvic chemotherapy and operation on pelvis, many hereditary diseases including absence of X chromosome, translocation between X chromosome and autosome, absence of gonadotropin or its receptor gene, mutations in FOXL2 gene and premutations in fragile X gene are considered correlating to POF. However, one-third to one-half of the cases are idiopathic and the identifiable aetiology of POF are still elusive. Ovarian biopsy of POF patients showed infiltration of lymphocytes and the characteristics of autoimmune oophoritis. Autoimmune ovarian disease (AOD) is considered one of the reasons leading to POF, but the immunological mechanism is still poorly understood due to short of specific clinical symptoms in the early stage of POF and lack of diagnostic features of ovarian inflammatory or autoimmunity disease. Auto-reactive T cell-mediated autoimmune oophoritis is characterized by the limited or diffused inflammation and accumulation of mononuclear cells in the stroma of ovary. Although ovarian inflammation does not affect the ovarian function, it can lead to infertility in female mice. T cell-mediated inflammation is the prerequisite in the pathogenesis of ovarian atrophy induction and infertility. Both of the follicular and interstitial regions can be involved, the interstitial inflammation may be restricted to the hilus of ovary, to the region around the follicles or to the entire ovary. Clinical manifestations are closely related with the range of damage, from asymptomatic ovarian inflammation to menstrual disorders, POF, and infertility.

The current study found that platelet-activating factor (PAF), a kind of phospholipid with a wide range of biological activity, can affect the disorder of cardiovascular, respiratory, digestive, reproductive, skin, and other tissues or systems through mediating inflammation. In order to ensure the role of PAF in inflammation, PAF receptor antagonist was involved in many researches in recent years, which can resolve and inactivate PAF and related phospholipids. PAF and PAF class oxidized phospholipids stimulate the occurrence and development of inflammation and atherosclerosis. PAF acetylhydrolase (PAF-AH) can hydrolyze PAF and PAF class oxidized phospholipids and eventually prevent the body from the damage of these inflammatory mediators.

It is currently well known that POF is closely related with autoreactive T cell-mediated autoimmune oophoritis. Since PAF and PAF-AH are linked with inflammation of ovary, it is wondered if there are any changes in the expression of PAF and PAF-AH in premature ovarian failure patient. How about the relationship between the expression of PAF and PAF-AH and the incidence of premature ovarian failure? Can we use PAF and PAF-AH as the future prediction factors of POF diagnosis and targets of POF treatment?
However, until now, there is no satisfied answer and further research in the field is required currently.

**PAF and biological effects of oxidized phospholipids**

In 1972, a kind of soluble phospholipid released by rabbit tropic alkaline granulocyte was reported by Benveniste, which was named PAF because of its platelet aggregation function [1]. Subsequent studies found that PAF is a mediator of inflammation with a wide range of biological activity and involved in various physiological and pathological processes [1].

PAF was produced by various cells such as neutrophils, basophils, macrophages, and vascular endothelial cells in many physiological or pathological conditions such as thrombosis, oxidative stress, and inflammation [1]. There are mainly two ways: one is the modified route, the hemolytic PAF (lyso-PAF) turn into PAF under the action of acetyltransferase. The other is the re-synthesis route, which is catalytic and synthesis in the basis of 1-O-alkyl-S non-glycerol phosphate-3, through acetyltransferase, phosphatase and phosphorylcholine transferase. Re-synthesis pathway is the main way under physiological conditions; the modified pathway is mainly involved in allergic reaction and other pathological reactions. PAF plays the biological role by activating G protein-coupled PAF receptors. It can cause platelet aggregation which can make neutrophils release oxygen free radicals, promote cell adhesion, and cause smooth muscle cells contract. It can also act on vascular wall endothelial cells to increase the permeability of vascular walls or emerge cascade amplifying function [2]. PAF receptors are widely distributed in human body, its increased generation or reduced degradation lead to the accumulation of PAF in body, and then its physiological role is magnified, which can cause pathologic situation. PAF participate in asthma, cerebral thrombosis, atherosclerosis, and the development of other diseases; it especially plays an important role in atherosclerosis.

PAF oxidized phospholipids, belonging to the super PAF family, is stimulated by oxidative stress. Because of similarity to PAF, PAF oxidized phospholipids can produce similar biological activity of PAF when combined with PAF receptor [3].

**Biological activity of PAF-AH**

PAF is synthesized and secreted by cells when stimulated, and then quickly eliminated through metabolism, which exist in vivo with dynamic equilibrium. The acetyl group at the sn-2 position of its glycerol backbone is essential for its biological activity and involves a variety of physiological responses. Deacetylation induces loss of activity of PAF. PAF-AH can hydrolyze the acetyl group on the Sn-2 position of PAF, which determine the level of PAF in the plasma and tissue. PAF-AH in the International Classification of the Commission as: EC3.1.47 belongs to hydrolase - esterase hydrolysis - carboxylic acid hydrolase class. The extensive research of PAF-AH began from about 1980, firstly in rabbits, then cattle and reptiles, and then fishes. PAF-AH has the following characteristics: 1) independent on Ca2+; 1) its substrate is acetyl group at the C2 position of glycerol backbone or short chain (including oxidated) fatty acids. This feature allows phospholipids and lipoproteins of the cell membrane not to be hydrolyzed and only hydrolyze the harmful substances such as PAF and oxidized phospholipids. PAF-AH belongs to phospholipase A2 family. It can be divided into two types in the body; one is plasma PAF-AH, also known as lipoprotein-associated phospholipase A2, which exist in the plasma in the form of lipoproteins. Plasma PAF-AH is primarily bound with low-density lipoprotein (low density lipoprotein, LDL) (L-PAF-AH) and about 20% is bound with high-density lipoprotein (high density lipoprotein, HDL) (H-PAF-AH). The other is intracellular PAF-AH, which can be divided into IB and IIB type. Different from other phospholipase A2, this type of PAF-AH has higher hydrolytic activity on the phospholipids with short-chain acyl ester at the sn-2 position. Therefore, it can specifically hydrolyze PAF and PAF-like oxidized phospholipids to induce the loss of biological activity, which determine the level of PAF and PAF-like oxidized phospholipids in plasma and tissue. In addition, in vivo/vitro experiments confirmed that the plasma-type PAF-AH can also regulate the inflammatory response by blocking the signal transduction commenced by PAF and PAF-like oxidized phospholipids.

**The relationship between PAF and disease**

PAF is a phospholipid with potent, diverse physiological actions which is involved in various physiological and pathological processes, such as asthma, sepsis, atherosclerosis, and hypertension [4-6]. When encountered with inflammation and oxidative stress, the level of PAF is increasing which is synthesized and secreted by macrophages, endothelial cells, neutrophils, and other cells that are involved in inflammation and vascular system [1], and then PAF exerts its biological activity by binding and activating G protein-coupled PAF receptor. PAF-like oxidized phospholipids is a class of oxidized phospholipids production that generated in the process of lipid oxidation and share the similar structure with the PAF and generate PAF-like biological action by binding with PAF receptor [7]. PAF and PAF-like oxidized phospholipids can increase the permeability of capillary blood vessels and the contraction of smooth muscle cells, and activate platelets, polymorphonuclear leukocytes, neutrophils macrophages, and other cells, and also have a strong pro-inflammatory action.

**The relationship between PAF-AH and inflammation and cardiovascular disease**

Endothelial injury and chronic inflammation play an important role in the occurrence and development of atherosclerosis, and even in the process of rupture at athero...
sclerosis site [8, 9]. Hyperlipidemia, particularly hyperlipidemia of increased oxidized LDL (Ox-LDL) can promote the development of atherosclerosis [10]. Because of their oxidation lipid and apolipoprotein (apo), Ox-LDL with pro-inflammatory reaction can activate white blood cells, platelets, and endothelial cells to produce the oxidation of lipids, including PAF. PAF can promote the inflammatory response and further the development of AS [11].

PAF and PAF-like oxidized phospholipids can induce the PAF-AH production [4, 5]. In 2000, Packard et al. first proposed that reduced PAF-AH, new coronary heart disease risk factors, can independently predict coronary events [7]. The expression of PAF-AH in the arterial wall of rabbit can reduce ox-LDL accumulation in the artery wall to achieve anti-inflammatory, antithrombotic, and anti-proliferation of smooth muscle cell [13]. The study found that the PAF-AH gene G994 mutation led to the reduction or loss of PAF-AH activity and the occurrence of coronary heart disease and cerebral infarction [7, 14]. Another study showed that activity of plasma PAF-AH in acute myocardial infarction decreased [14]. Recent study indicated LDL in the patients with PAH-AH defects is more prone to oxidation comparing with control group and has a stronger role in stimulation of adhesion molecules [15]. These studies suggested that plasma PAH-AH has the role of anti-inflammatory, anti-oxidation, and anti-atherosclerosis [11, 12].

Recently, however, some studies found that concentration and activity of PAF-AH increased in the patients with coronary heart disease. A large case-control study in Europe found that patients with decreased activity of PAF-AH accompanying V397 homozygous alleles have low coronary heart disease risks, which suggested that PAF-AH has a direct role in promoting atherosclerosis [16]. These studies suggested that lysophosphatidylcholine (lyso-PC), oxidized free fatty acids (OX-FFA) and other substances, which are produced by PAF-AH hydrolysis on PAF and PAF-like oxidized phospholipids, are pro-inflammatory mediators, can stimulate the production of adhesion molecules and cytokine, and promote the formation of atherosclerosis [7]. Considered together, the impact of PAF-AH on the atherosclerosis and cardiovascular disease is dual [17, 18]. Chen proposed that the advantages and disadvantages of the PAH-AH in plasma affecting on the body depends on the final effects of PAF-AH hydrolysis [8].

More and more studies have shown that the increase of LDL-PAF-AH activity is risk factors of atherosclerosis and cardiovascular disease [3]. Oxidative stress increased plasma levels of Ox-LDL, LDL-PAF-AH activity and lyso-PC, which may have a negative impact on the body. PAF-AH is stored in the body as HDL-associated PAF-AH which can degenerate extra PAF or PAF-like oxidized phospholipids [19]. HDL-associated PAF-AH plays a protective role against inflammation and oxidation and is considered a protective factor against atherosclerosis [5]. With adenovirus-mediated gene transfer of PAF-AH in ApoE-deficient mice, the level of oxidized LDL decreased, however, HDL-associated plasma PAF-AH and PAF-AH activity in plasma increased, and vitro antioxidant of various lipoproteins also increased. In addition, the ability that HDL promotes cholesterol spilling from cultured macrophage and inhibits foam cell formation was enhanced [20].

Some studies found that in the cholesterol-fed rabbits, PAF-AH activity in plasma increased through augmenting the secretion of PAF-AH activity from macrophages. Meanwhile, lyso-PC was increased. Simvastatin treatment reduces PAF-AH and LDL-associated PAF-AH activity in plasma in the cholesterol-fed rabbits by decreasing the level of plasma LDL [14]. In the patients with familial hypercholesterolemia, both PAF-AH and LDL-associated PAF-AH activity were increased, while HDL-PAF-AH/LDL-PAF-AH ratio was significantly decreased [7]. Caslake and Packard stated that the increase of HDL-PAF-AH / LDL-PAF-AH ratio may contribute to preventing the formation of atherosclerosis [17].

The relationship between PAF-AH and POF

Plasma PAF-AH, especially HDL-associated PAF-AH, may be a hydrolytic enzyme against inflammation, oxidative stress and atherosclerosis [21]. 1/3~ 1/2 of the POF are idiopathic. Studies have shown that idiopathic POF was related with autoimmune oophoritis. The inflammation of ovaries induced the damage of ovarian tissue, consequently affecting follicular development elicit follicular atrophy, finally leading to POF. In addition, inflammation leads to the collection of inflammatory mediator PAF and the increase of relevant PAF-AH. Because PAF-AH, as receptor antagonist of PAF, can transfer PAF to inactivated form, hence the relationship of PAF-AH and PAF is converse. If PAF-AH is not sufficient to be against PAF; the increase of PAF might lead to pathological change of ovaries through excessive accumulation of PAF. The hypothesis of treatment of POF through improving the level of PAF-AH in patients to eliminate the accumulation of PAF in the ovarian tissue needs more laboratory data to confirm. Plasma PAF-AH, especially HDL-associated PAF-AH, may be a hydrolytic enzyme against inflammation, oxidative stress, and atherosclerosis [21]. One-third to one-half of the POF are idiopathic. Studies have shown that idiopathic POF is related with autoimmune oophoritis. The inflammation of ovaries induce the damage of ovarian tissue, consequently affect follicular development, elicit follicular atrophy, and finally leading to POF. In addition, inflammation leads to the collection of inflammatory mediator PAF and the increase of relevant PAF-AH. Because PAF-AH, as receptor antagonist of PAF, can transfer PAF to inactivated form, the relationship of PAF-AH and PAF is converse. If PAF-AH is not sufficient to be against PAF, the increase of PAF might
lead to pathological change of ovaries through excessive accumulation of PAF. The hypothesis of treatment of POF through improving the level of PAF-AH in the patients to eliminate the accumulation of PAF in the ovarian tissue needs more laboratory data to confirm.

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References


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e-mail: liangzxu@126.com
Behcet disease and pregnancy

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Summary

Purpose: Behcet disease (BD) is a multisystemic vaculitis commonly diagnosed in reproductive years. The authors aimed to investigate the relationship between BD and pregnancy outcomes. Materials and Methods: In this multicenter retrospective survey study, the authors compared the pregnancy outcomes of BD patients with the healthy controls. Results: A total of 298 pregnancies of 94 patients with BD and 219 pregnancies of 95 healthy controls were evaluated. The mean birth weight of all babies of women with BD and the control group were 3,214 grams and 3,351 grams, respectively ($p = 0.028$). The miscarriage rates were also higher in the BD group. The complication rates of pregnancy with hypertension, preeclampsia, preterm labour in the study group and the control group were 12.8% and 11.6%, respectively ($p = 0.489$). Conclusion: The current study demonstrated that BD patients delivered smaller babies and they have higher miscarriage rates when compared to the healthy controls which might be due to the vasculitis of the placenta.

Key Words: Behcet Disease; Pregnancy outcome; Fetal outcome; Vasculitis.
summarized in Table 2. Mean age of diagnosis of BD in the study group was 32.29 ± 9.43 years.

Episiotomy infection was seen in two women with BD and transient neonatal lesions were seen in the babies of five women but none of them required treatment for the baby.

From 94 BD patients, 74 of them (78.7%) had no changes, 18 of them (19.2%) had exacerbation, and two of them (2.1%) had remission during the pregnancy.

The comparison of clinical manifestations of BD according to the menstrual cycle significantly correlated with clinical manifestations during the pregnancy. If a woman had no changes during menstruation it might help to predict that she would not have changes in clinical manifestations during the pregnancy.

Complication ratios of pregnancy with hypertension, preeclampsia, preterm labour in the study group, and the control group were 12.8% and 11.6%, respectively (p = 0.489). Still birth ratios were not significantly different between groups either.

### Discussion

In the current study, 298 pregnancies in 94 women with BD were compared with 219 pregnancies in 95 healthy controls and it was demonstrated that BD increased the miscarriage rates and birth weight when compared with the healthy controls, but BD did not increase the pregnancy complications. Pregnancy usually had no influence on the symptoms of BD.

Marshal et al. [4] evaluated 61 pregnancies in 23 women retrospectively. They compared them with 83 pregnancies of 30 women with recurrent oral ulcers and 61 pregnancies of 20 healthy women. They investigated the pregnancy complications, the fetal outcomes, and BD course during the pregnancy and the postpartum period. They found no significant difference among groups on the pregnancy complications and the fetal outcomes. The only serious complication was the Budd-Chiari Syndrome of a patient in the puerperium which might be coincidental, one patient had oral ulcer exacerbation, and one had recurrent episiotomy infection in the study group. They report no neonatal BD from 55 newborns in the study group. They concluded that even they had a small sample size perinatal outcome of BD patients were good, the clinical manifestation of the disease was not worsened by the pregnancy.

Jadaon et al. [5] evaluated 135 pregnancies of 31 BD women in their retrospective study. Mean age of the diagnosis was 24.48 ± 8.84 years. Mean parity at the diagnosis was 4.35 ± 2.66. They formed an age, parity, and ethnic origin that matched the control group to compare the study group. When comparing the pregnancies of the same patients before and after BD diagnosis, the miscarriage ratios did not differ but the pregnancy complications increased after the diagnosis of BD. They showed that remission was more prevalent than exacerbation during the pregnancy and patients tended to continue in the same direction after the delivery. They found that the pregnancy complications were more prevalent in BD group when compared to the control group. Neonatal outcomes including birth weight was not different between groups. They concluded that while maternal outcomes adversely affected from BD, neonatal outcomes did not change when compared with the control group. Remissions were five times more prevalent than exacerbations during the pregnancy and puerperium. They concluded that the pregnancy had no adversity but positive effects on BD, on the other hand, BD might have some adverse effects on the pregnancy outcomes including higher miscarriage rates [5].

The present authors demonstrated that neonatal birth weight was significantly lower in BD group unlike Jadaon et al. [5]. This difference might be because of the small sample size of the previous study. Like the previous studies [5], the miscarriage rates were higher in BD group in the current study.

In a study conducted in 2008 placentas of two patients were evaluated microscopically and it was demonstrated that both first trimester and term placentas of BD patients showed necrotizing villitis and neutrophil dominant infiltration. The authors excluded the infectious diseases both clinically and histologically [6]. As a result, higher miscarriage rates and lower birth weights in BD group in the current study can be explained by the vasculitis of the placenta. Even when a patient conceives in remission period, treatment might be used to decrease the miscarriage and the low birth weight rates especially in those who have poor obstetric history.

### Table 1. — Distribution of the patients according to the hospitals.

<table>
<thead>
<tr>
<th>Institution</th>
<th>Behcet’s group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tokat University</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Canakkale University</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Hatay University</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Afyon University</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Ankara Kecioren Hospital</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Elazig University</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>95</td>
</tr>
</tbody>
</table>

### Table 2. — Demographic characteristics and obstetric outcomes of the groups.

<table>
<thead>
<tr>
<th></th>
<th>BD</th>
<th>control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.65 ± 10.03</td>
<td>40.07 ± 10.09</td>
<td>0.778</td>
</tr>
<tr>
<td>Gravida</td>
<td>3.17 ± 1.44</td>
<td>2.30 ± 1.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parity</td>
<td>2.56 ± 1.06</td>
<td>1.93±0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abortus</td>
<td>0.55 ± 0.87</td>
<td>0.33±0.61</td>
<td>0.05</td>
</tr>
<tr>
<td>Ectopic pregnancies</td>
<td>0.05±0.30</td>
<td>0.03±0.17</td>
<td>0.552</td>
</tr>
<tr>
<td>Birth weight of first newborn (grams)</td>
<td>3,153 ± 575</td>
<td>3,373 ± 541</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean birth weight of all newborns (grams)</td>
<td>3,214 ± 416</td>
<td>3,351 ± 428</td>
<td>0.028</td>
</tr>
</tbody>
</table>
The course of BD during pregnancy changes among patients even in the different pregnancies of the same patient. Hamza et al. [7] evaluated 21 pregnancies of eight patients and while remission were seen in 12 pregnancies, exacerbations were seen in nine pregnancies. All exacerbations were seen in the dermatological manifestations such as erythema nodosum, genital aphthosis, necrotic pseudofolliculitis, and bucchal aphthosis. In the retrospective study of Uzun et al. [8] the authors concluded that pregnancy did not affect BD markedly and BD did not increase the complications of pregnancy after analyzing 44 pregnancies of 28 patients. Jadaon et al. reviewed the literature and concluded that remission rate was 56.6% (83 pregnancies) and exacerbation rate was 35.6% (51 pregnancies) during pregnancy [5]. Bang et al. [9] demonstrated higher exacerbation ratio in their study. They evaluated 27 pregnant patients and demonstrated that the majority of exacerbations occurred in the first trimester. An interesting finding of the authors was that exacerbation was seen commonly in the mucocutaneous manifestations when compared with other types. Majority of our patients had neither remission nor exacerbation in the present study.

In a recent study Zhang et al. analyzed 334 BD patients and concluded that the mean age of onset was 35.8 ± 11.1 years [10] which was concordant with the present study.

Neonatal BD is a very rare condition and almost always seen with the active maternal disease. It is manifested with oral ulcers, skin lesions, fever, and leukocytosis [11]. In the current study only five babies showed transient skin lesions which did not need to be treated.

The main factor that strengthens the present study is the large sample size. To the authors’ knowledge, it is the largest sized study in the English literature. The main limitation of this study is its retrospective design. Because BD is a relatively rare disease and follow-up period must be very long, conducting a prospective study is not efficient. Previous studies were also performed in a retrospective manner. Because it is sometimes difficult to remember the specific symptom of a chronic disease or the treatment taken in a particular time like pregnancy, the authors only asked the general exacerbation or remission of the disease during pregnancy. Nearly every woman remem-
Proteomic investigation of the severe preeclampsia treatment by low molecular weight heparin

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Summary

Purpose: The primary goal of this study is to investigate the mechanism of severe preeclampsia (PE) treatment by low molecular weight heparin (LMWH). Materials and Methods: Using two-dimensional difference in-gel electrophoresis (2D-DIGE) combined with matrix assisted laser desorption ionization-time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF) approach to identify the proteins that expressed differently in the serum samples of five patients before and after subcutaneous injection of LMWH (0.4 ml/person). Results: Seven protein spots were identified in 2D-DIGE that show significant change in expression level after LMWH treatment. Further analysis of seven protein spots with MALDI-TOF/TOF identified six different proteins. To confirm the proteomic data, two meaningful proteins of the six proteins, alpha-1-acid glycoprotein (AGP) and serotransferrin are subjected to immunoblotting. All of the proteins are obviously down-regulated after LMWH treatment. Conclusions: PE is a pregnancy-specific disease that clinically manifests as new-onset hypertension and proteinuria after 20 weeks of gestation. LMWH is an effective treatment of severe PE. The present proteomics based investigation may provide a new angle to understand the mechanism of severe PE treatment with LMWH.

Key words: Severe preeclampsia; LMWH; DIGE; MALDI-TOF/TOF; Human serum.

Introduction

Preeclampsia (PE) is a systemic syndrome that occurs in 3% ~ 5% of pregnant women worldwide [1, 2]. It is estimated that each year more than 500,000 women die of pregnancy related diseases, and 10% ~ 15% of maternal deaths are associated with PE and eclampsia; 99% of fatalities occurs in low- and middle-income countries [3]. PE is a pregnancy-specific disease characterized by development of concurrent hypertension and proteinuria, which sometimes progresses into multiple organ cluster with varying clinical features [4]. Despite numerous clinical investigations and intensive researches, pathophysiological mechanisms of this disease still remain unknown [5]. As a result, the development of preventive and therapeutic treatments falls further behind [6]. Placental perfusion insufficiency caused by micro thrombus plays a crucial role in the development of PE [7]. Preliminary nonrandomized studies suggest some benefit from adjuvant therapy with anticoagulation by means of LMWH in PE [8-12]. LMWH is an anticoagulant drugs that can prevent and reduce thrombus including placental micro thrombus. Because LMWH does not cross the placental barriers, it does not cause fetal teratogenicity [13]. The goal of this study is to identify proteins markers that may explain the mechanism of adjuvant treating severe PE with LMWH.

Proteomic techniques play an important role in the study of changes in proteomes expression of patients serum samples [14]. Identification of these proteins can thus significantly increase the availability of molecular markers for early diagnosis and therapy, leading to better understanding the mechanisms of LMWH adjuvant treatment for PE [15]. Two-dimensional electrophoresis (2D-E) is extensively used in proteomics to compare changes in protein expression [16, 17]. Although proteomic studies of the PE are performed in the past, they are mostly limited to the samples acquired from placenta rather than from serum [18-21]. In addition, none of those studies focused on the difference between untreated and LMWH treated patients of severe PE. Thus a comparative study is required to gain insights into the mechanism of PE adjuvant treatment by LMWH.

Two-dimensional differential in gel electrophoresis (2D-DIGE) was first described by Unlü et al. as a technique to profile proteins [22]. 2D-DIGE is a method that labels protein samples with different fluorescent dyes before 2D electrophoresis, and then separates three different protein samples at the same time in one two-dimensional gel. The application of the internal standard ensures that the results reflect the real biological differences, and in the meantime avoids influence of systematic errors [23-26]. The most important advantage of the DIGE system lies in that it integrates CyDye DIGE dye multiple labeling method into DeCyder difference 2D analysis software. DeCyder software takes the advantage of the spots co-detecting algorithm, which automate the entire process, including acquisition of fluorescence images, background elimination, quantification, normalization, and matching spots in gel. As a result, operational errors are minimized. Therefore, it is an ideal tool to obtain the information of differential proteome in samples of the patients with severe PE before and after LMWH treatment.
Materials and Methods

Subjects and serum sample collection

Samples were collected at the Department of Obstetrics and Gynecology, NanFang Hospital. The clinical characteristics of patients are summarized in Table 1. Blood was drawn from the peripheral vein and serum was produced by centrifugation. Serum samples in Group 1 were taken from five pregnant women with severe PE. These patients did not receive any treatment before being admitted to NanFang hospital. Samples in Group 2 were taken from the same five patients, after receiving 0.4 ml LMWH subcutaneous injection. Since the peak concentration in blood of LMWH usually appears in six hours after injection, blood samples were drawn at eight to ten hours after LMWH injection. Table 1 shows the information of five patients in age, body mass index (BMI), gestational age, systolic blood pressure (SBP) or diastolic blood pressure (DBP).

Severe PE was diagnosed as follows: blood pressure ≥ 160/110 mmHg, proteinuria ≥ 2+ (two or more episodes, two measurements ≥ six hours apart) or proteinuria ≥ 2 grams in 24 hours along with any of the following: platelets < 100,000/ml, elevated aspartate aminotransferase or aminotransferase levels, elevated lactate dehydrogenase levels, creatinine > 106 μmol/l, persistent headache, central nerve dysfunction, blurred vision, or persistent epigastric discomfort. All of the subjects’ blood pressure normalized 12 weeks after delivery.

All of the five participants signed verbal informed consent prior to sample collection. The Ethics Committee of NanFang Hospital approved the research protocol. The screening criteria excluded patients who had: 1) PE history; 2) chronic hypertension before the indexed pregnancy; 3) other metabolic diseases such as hyperthyroidism or hypothyroidism, diabetes or gestational diabetes, nephropathy, and polycystic ovary syndrome; 3) other diseases such as congenital heart diseases; and 4) addiction to smoking or alcohol.

Samples preparation for 2D-DIGE analysis

Serum samples (five ml each) were extracted from the peripheral blood by centrifuging at 5,000 rpm for ten minutes at 4°C within 30 seconds of collection and then stored at -80°C. Precise kits were used to specifically remove albumin and IgG, followed by desalting and concentration with another clean-up kit. Protein concentration of supernatant is measured with the Precise kits were used to specifically remove albumin and IgG, and 4% (w/v) CHAPS.

2D-DIGE and image analysis

The internal standard was labeled with a cyanine 2 dye and equal amount of protein was loaded on each gel to control the gel-gel variation. The pre-treatment sample was labeled with Cy3, an equal amount of protein was loaded on each gel to control the gel-gel variation. Quantitative differences were only accepted when at least a 1.5-fold change was confirmed.

Table 1. — Demographic information of the pregnant women included in this study.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Maternal age (y)</th>
<th>Gestational weeks (w)</th>
<th>BMI (kg/m²)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.7</td>
<td>36+4</td>
<td>28.07</td>
<td>168</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>25.8</td>
<td>36+1</td>
<td>29.11</td>
<td>170</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>27.1</td>
<td>35+6</td>
<td>27.93</td>
<td>169</td>
<td>114</td>
</tr>
<tr>
<td>4</td>
<td>26.2</td>
<td>36+2</td>
<td>28.85</td>
<td>178</td>
<td>116</td>
</tr>
<tr>
<td>5</td>
<td>27.4</td>
<td>35+5</td>
<td>27.69</td>
<td>171</td>
<td>113</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using air at collision energy of 2 kV and MS analysis, 1,000 shots were typically accumulated. MS/MS analysis was performed using air at collision energy of 2 kV. MASCOT search engine (version 2.1) was used to search all of the tandem mass spectra. GPS software (version 3.6.2) was used to create and search files with the MASCOT search engine for protein identification. Protein identities were obtained by using the MASCOT search engine against non-redundant databases selected for human taxonomy.

In-gel digestion and protein identification

Proteins were identified by peptide mass fingerprinting (PMF) from spots that are digested from preparative gels stained with SYPRO Ruby. Protein spots were then excised from the 2D gel. The spots were in-gel digested with trypsin following Bruker’s standard in-gel digestion protocol and stored at -20°C.

Samples on the MALDI target plates were then analyzed using a proteomics analyzer MALDI-TOF/TOF mass spectrometer. For MS analysis, 1,000 shots were typically accumulated. MS/MS analysis was performed using air at collision energy of 2 kV. MASCOT search engine (version 2.1) was used to search all of the tandem mass spectra. GPS software (version 3.6.2) was used to create and search files with the MASCOT search engine for protein identification. Protein identities were obtained by using the MASCOT search engine against non-redundant databases selected for human taxonomy.

Western blot analysis

Two differentially expressed proteins, alpha-1-acid glycoprotein (AGP) and serotransferrin (TF), were further validated by Western blot using the ubiquitous protein β-Actin as the loading control. Equal amount of total protein (20 μg) extracted from the individual serum samples was resolved in 12% SDS-PAGE. Proteins were electrophoretically transferred to nitrocellulose membranes. The membranes were blocked for one hour at room temperature in TBST (20 mM Tris-HCl pH 7.6, 140 mM NaCl, pH 7.5, 0.05% Tween-20) containing 5% skim milk before the mouse monoclonal primary antibodies were added. Membranes were incubated with each primary antibody at room temperature for two hours. Blots were then washed for five times, incubated with the dylight flour conjugates labeled polyclonal goat anti-mouse IgG (diluted 1:10000) for one hour at room temperature. After being washed five times with TBST, immuno-reactive complexes were visua-
Proteomic investigation of the severe preeclampsia treatment by low molecular weight heparin

Figure 1. — Standard curve for protein quantification using 2-D Quant kit.

Figure 2. — Albumin and IgG were effectively removed from the serum samples. A: total protein of one serum sample. B: albumin/IgG-depleted serum fraction. C: the eluted fraction from albumin removal column.

Table 2. — Differentially expressed proteins identified in PE from two groups by DIGE and MALDI-TOF/TOF.

<table>
<thead>
<tr>
<th>Spots Position</th>
<th>Protein description</th>
<th>Accession No.</th>
<th>Protein Score</th>
<th>C.I.%</th>
<th>Protein MW (kDa)</th>
<th>Protein PI</th>
<th>Pep. count</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-209</td>
<td>Alpha-2-macroglobulin</td>
<td>Sp</td>
<td>P01023</td>
<td>A2MG_HUMAN</td>
<td>155</td>
<td>100</td>
<td>164,6004</td>
</tr>
<tr>
<td>486</td>
<td>Serotransferrin</td>
<td>Sp</td>
<td>P02787</td>
<td>TRFE_HUMAN</td>
<td>56</td>
<td>99.99</td>
<td>79,2805</td>
</tr>
<tr>
<td>606</td>
<td>Serum Albumin</td>
<td>Sp</td>
<td>P02768</td>
<td>ALBU_HUMAN</td>
<td>52</td>
<td>100</td>
<td>71,3172</td>
</tr>
<tr>
<td>1182</td>
<td>Alpha-1-acid glycoprotein</td>
<td>Sp</td>
<td>P02763</td>
<td>A1AG1_HUMAN</td>
<td>130</td>
<td>100</td>
<td>23,7248</td>
</tr>
<tr>
<td>1604</td>
<td>Hemoglobin Subunit beta</td>
<td>Sp</td>
<td>P6887I</td>
<td>HBB_HUMAN</td>
<td>168</td>
<td>100</td>
<td>16,1023</td>
</tr>
<tr>
<td>1616</td>
<td>Hemoglobin Subunit Alpha</td>
<td>Sp</td>
<td>P69905</td>
<td>HBA_HUMAN</td>
<td>224</td>
<td>100</td>
<td>15,305</td>
</tr>
</tbody>
</table>
Shuoshi Wang, Shuiwang Hu, Mei Zhong

ized using ECL reagents and imaged with X-ray films that was later scanned. A semi-quantitative analysis, which was basing on OD was performed by a specific software.

Results

Subject characterization
Five pregnant women complicated with severe PE are recruited in this study. After received cesarean section in their trimester to terminate the pregnancy, their blood pressures dropped to the normal range. The characteristics of these pregnant women are listed in Table 1, which shows the information regarding their maternal age, gestational weeks, BMI, SBP or DBP.

Serum sample preparation
Accurate quantification of proteins is necessary for DIGE. Figure 1 shows the standard curve generated by the 2-D quant kit. To increase the resolution, albumin and IgG, two major plasma proteins of the human were removed (Figure 2) prior to electrophoresis, and no significant difference in SDS-PAGE was observed between the samples of the two sample groups (Figure 3).

Protein identification
The identification of proteins differentially expressed in Group 1 and Group 2 was performed by DIGE. Figure 4 shows the spots of differentially expressed proteins between serum samples. Seven spots were detected using the image analysis software (Figure 5). Spots with a fold change equal or above 1.5 (in absolusion value) were exclusively considered as differentially expressed spots. Spots shown in Fig. 4 were selected for further identification using a MALDI-TOF/TOF mass spectrometer. As a result, six different proteins were identified differentially expressed between Group 1 and Group 2, among which alpha-1-acid glycoprotein (AGP) and serotransferrin (Tf) may have physiological significance. All six proteins were down-regulated after LMWH subcutaneous injection (Table 2).

Protein validation
Proteins showing significantly different expression after LMWH are likely to have biological impact. To further validate the DIGE results, two proteins, AGP and Tf were selected for Western blots base upon their biological functions, high fold changes and the availability of commercial antibodies. At 42 kDa and 80 kDa, antibodies identified the bands for AGP and Tf, respectively (Figure 6). Both AGP and Tf were detected as strong bands in the samples from Group 1, while weak signals were observed in the samples from Group 2, agreeing with the DIGE results (Figure 6). In conclusion, the Western blots confirmed the down-regulation of AGP and Tf after LMWH treatment.

Discussion
In this study the authors have demonstrated a possible molecular mechanism for adjuvant treating PE with LMWH. The proteins identified in this report could also serve as potential biomarkers for the diagnosis and treatment of PE.
As a systematic syndrome primarily manifested by hypertension and proteinuria and mainly seen in the second half of pregnancy, PE affects approximately 3% to 5% of pregnancies worldwide. [27] PE was the leading cause of maternal mortality, preterm birth, and consequent neonatal morbidity and mortality. In developing countries where access to safe, emergency delivery is inadequate, PE claims the lives of more than 60,000 women every year. [28] Exciting progress has been made in pathophysiology of PE recently. However the exact cause of this disease remains unknown. For this reason, it is difficult to develop treatments or preventive strategy.

In this study, the authors have identified serum proteins that significantly down-regulated by LMWH treatment. The techniques of 2D-DIGE and MALDI-TOF/TOF allow proteomic studies and make it possible to unveil new pathophysiological mechanisms and to identify new biomarkers and/or potential therapeutic targets.

Totally, six different proteins were found. They are alpha-1-acid glycoprotein, serotransferrin, serum albumin, alpha-1-acid glycoprotein, hemoglobin subunit alpha, and hemoglobin subunit beta. As known, there are some physiological proteins in human bodies and these kinds of proteins participate in almost every physiological activity but not in special functions. After analysis, the authors believe the possible functions proteins are AGP and Tf. Both of them are discussed below.

**AGP (alpha-1-acid glycoprotein)**

AGP was originally described in 1950 [29-31] and later characterized as a very unusual protein with a very low pI (in the range from 2.8 to 3.8) and a very high carbohydrate content (45%) [32]. Human AGP is the product of a cluster of three adjacent genes: AGP-A, AGP-B and AGP-C, which covers 70 kb on chromosome 9 [33, 34]. AGP is considered as a natural anti-inflammatory and immuno-modulatory agent because of its anti-neutrophil and anti-complement activity [35]. AGP was reported to act in vitro and in vivo as an immuno-modulating molecule. In vitro, AGP inhibits polymorph nuclear neutrophil activation [36], increases the secretion of an IL-1 inhibitor by murine macrophages and most probably the IL-1 receptor antagonist [37, 38], and modulates LPS-induced cytokine secretion by monocytes macrophages [39].

First, it is extensively realized that the inflammatory factor and disimmunity are responsible for many cases of PE. After treating with LMWH, the level of AGP was reduced. A possible explanation is that LMWH inhibits inflammatory factors, helps alleviate the syndrome of severe PE, especially in the acute phase, which in turn reduces the level of AGP. Second, human serum albumin, lipoprotein, and AGP are most important drug binding proteins in plasma that can have important pharmacokinetic implications [40]. The variations in AGP levels during inflammatory processes can considerably change the level of free drug without affecting its total plasmatic concentration. Therefore, the free concentration of the drug in plasma will reflect more accurately the intensity of the pharmacological effect. In the present experiment, AGP was down-regulated, probably because AGP bound with LWMH and facilitated the transportation of LMWH to other organs. This may result in a reduced concentration of AGP in plasma.

In summary, AGP is an acute phase protein. Its plasma levels can be used as a diagnostic and prognostic indicator during clinical therapy. The large variation observed in the binding ratios of basic drugs in plasma during several physiological and pathological states could be correlated with the large variations in the plasma level of AGP.

**Tf (Serotransferrin)**

Tf is a ubiquitous protein with a central role in iron transport and metabolism. During gestation, both the mothers and fetus require large amounts of iron that comes mainly from Tf [41]. It transports iron from sites of absorption and degradation to those of storage and utilization [42]. Tf is a bilobal 80 kDa iron binding glycoprotein. The single-chain polypeptide folds into two homologous lobes (N- and C-lobes) connected by a short peptide linker. Each lobe is further divided into two subdomains that come together to form the metal binding cleft [43].

There is some evidence showing that Tf has many other biological functions in addition to facilitating iron transport and metabolism. These functions may have profound effects on mammalian cell growth and productivity. The multiple functions of Tf can be exploited to develop many novel applications [44-50]. Significant progresses have been made towards expanding Tf applications in biotechnology and medicine. The effort is well-received for its potential in new therapeutic strategy for disease modification or treatment as well as a novel carrier system for targeted drug delivery [51].

Targeted drug delivery is another intensively studies area nowadays in pharmaceutical and biotechnological research and development. It is broadly defined as the selective delivery of a drug to specific biological site (tissue or cell). Among a large variety of drug delivery systems, ligand-receptor-mediated delivery systems have received considerable attention. From this perspective, Tf or its receptor may be useful as a targeting ligand to achieve targeted delivery of therapeutic agents in the treatment of PE [52]. In the present proteomic results, the down-regulation of Tf in LMWH treated samples implies that it may function in the transportation or targeted drug delivery of the LMWH.

As a proof of concept, this study is deemed to be an early effort on understanding the role of LMWH treatment for PE. Two proteins were identified by 2D-DIGE and MALDI-TOF/TOF and further verified by Western blots. Further studies focusing on the functional properties of these proteins may lead to the identification of protein biomarkers for early diagnosis and to the development of new diagnostic methods.
Acknowledgements

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Proteomic investigation of the severe preeclampsia treatment by low molecular weight heparin


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Effectiveness of ultrasound-guided transversus abdominis plane block and rectus sheath block in pain control and recovery after gynecological transumbilical single-incision laparoscopic surgery

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Summary

Purpose: To evaluate the effectiveness of ultrasound-guided transversus abdominis plane (TAP) and rectus sheath (RS) blocks in pain management and recovery after gynecological single-incision laparoscopic surgery (SILS). Materials and Methods: A bilateral TAP block (Group A, n=9), bilateral TAP and RS blocks (Group B, n=10), and a bilateral RS block (Group C, n=9) with 40 ml ropivacaine per patient were conducted in 28 patients undergoing SILS for ovarian tumors. A pain score and walking distance in a 6-minute walk test (6MWT) were examined. Results: Pain scores were significantly lower on postoperative day (POD) 3 than on POD 1 in Groups B (p = 0.03) and C (p = 0.02). The walking distance on POD 3 was comparable with that before surgery in Group C (p = 0.75), but shorter in Groups A (p = 0.004) and B (p = 0.02). Conclusions: The RS block alone was the most effective in relieving pain and accelerating general recovery after gynecological SILS.

Key words: Single-incision laparoscopic surgery; Transversus abdominis plane block; Rectus sheath block; 6-minute walk test.
Following the induction of general anesthesia, a TAP block with 40 ml of 0.375% ropivacaine (Group A), a TAP block with 20 ml of 0.375% ropivacaine and an RS block with 20 ml of 0.375% ropivacaine (Group B), and an RS block with 40 ml of 0.375% ropivacaine (Group C) were administered to 9, 10, and 9 patients, respectively (Figure 1). The authors used a real-time, in-plane needle insertion technique under US guidance. The TAP and RS blocks were administered under US guidance with a portable US device and a linear 6–13-MHz US transducer. The blocks were administered with a 22-G, 80-mm Tuohy nerve block needle. The TAP block was administered using a mid-axillary approach. After visualization of the external oblique abdominal muscle (EOAM), internal oblique abdominal muscle (IOAM), and transverse abdominal muscle (TAM) at the level of the mid-axillary line between the 12th rib and the iliac crest, the puncture area and a US probe were prepared in a sterile manner. After placing the tip of the needle in the space between the IOAM and TAM and confirming negative aspiration of blood, the TAP block was bilaterally administered by infusion of 20 ml of 0.375% ropivacaine per side in Group A and ten ml of 0.375% ropivacaine per side in Group B (Figure 1). For patients randomized to receive the RS block, the area of the abdomen between the lateral border of the rectus muscle and one cm cephalad to the umbilicus and a US probe were prepared in a sterile manner. The needle tip was placed close to the lateral border of the rectus sheath between the posterior sheath and rectus muscle. Spread of the local anesthetic was visualized between the rectus sheath and rectus abdominis muscle under US guidance (Figure 2). The RS block was bilaterally administered by infusion of 10 ml of 0.375% ropivacaine per side in Group B and 20 ml of 0.375% ropivacaine per side in Group C.

Anesthesia was maintained by propofol and remifentanil titrated to maintain the mean arterial blood pressure at 80%–120% of that before the induction of anesthesia. The propofol dose was adjusted to maintain the BIS between 40 and 60 during surgery. Following skin closure, 400 mg of acetaminophen was administered by suppository and anesthesia was discontinued before tracheal extubation. To manage postoperative pain, the authors used...
Effectiveness of ultrasound-guided transversus abdominis plane block and rectus sheath block in pain control and recovery after etc.

Assessment

The sites of administration of the TAP and RS blocks were visually checked for the presence of hematoceles or infection. The total amount of remifentanil and propofol administered was recorded. The presence or absence of nausea and vomiting after extubation and during the first 24 hours after surgery were recorded for each patient.

Sedation effects were evaluated using a numerical rating scale (NRS) and the 6-minute walk test (6MWT). NRS was used to assess pain intensity in patients who were able to self-report. All patients were assessed on a 11-point scale numbered from 0 to 10 (high scores indicating intolerable pain) on PODs 1 and 3.

The 6MWT is used as a performance-based measure of functional exercise capacity and exercise tolerance [14]. In this study, it was performed indoors on a hard, level surface in a straight corridor, free of distractions and in accordance with a standardized protocol recommended by the American Thoracic Society. Standard phrases of encouragement at one-minute intervals were provided during the 6MWT to control the influence of encouragement on test performance [15]. Participants were required to walk as far as possible in six minutes, but they were allowed to stop and rest as required. To ensure patient safety, blood pressure was measured immediately before and after the 6MWT, while heart rate and peripheral oxygen saturation (SpO2) were monitored continuously by finger pulse oximetry. A resting blood pressure of >150/100 mmHg and/or a heart rate of >100 beats per minute (bpm) precluded the 6MWT. The postoperative 6MWT was interrupted if SpO2 dropped to below 90% or if the heart rate exceeded 125 bpm. The 6MWT was conducted once before surgery to establish a baseline and again on PODs 1 and 3.

Statistical analysis

Differences in baseline demographics among the three groups were expressed as average values ± standard deviations, and one-way analysis of variance (ANOVA) was used to evaluate these differences.

The frequency of administration of flurbiprofen and loxoprofen sodium hydrate was expressed as medians (range), and differences in values for OPD and PODs 1–3 among the three groups were compared using the Kruskal–Wallis test. The NRS scores for postoperative pain were expressed as medians (range). Differences in scores between PODs 1 and 3 in each group were compared using the Wilcoxon signed rank test, and differences among the three groups on PODs 1 and 3 were compared using the Kruskal–Wallis test.

All patients successfully underwent a transumbilical SILS procedure without open conversion. No complications associated with the block procedures were encountered. All patients satisfied the authors’ well-defined discharge criteria and were discharged to their homes on POD 5. There were no statistically significant differences with regard to age, body height and weight, body mass index, duration of anesthesia and surgery, and dose of remifentanil and propofol among the three groups (Table 1).

The frequency of intravenous administration of flurbiprofen was 1 (0–2), 1 (0–2), and 1 (0–2) on OPD and PODs 1–3 in Groups A, B, and C, respectively. The frequency of

Table 1. — Baseline demographics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient number</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Anesthesia time (min)</th>
<th>Operation time (min)</th>
<th>Propofol (mg)</th>
<th>Remifentanil (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>9</td>
<td>32±6</td>
<td>159±5</td>
<td>54±7</td>
<td>21.1±2.4</td>
<td>129±23</td>
<td>79±24</td>
<td>611±137</td>
<td>1.4±0.5</td>
</tr>
<tr>
<td>Group B</td>
<td>10</td>
<td>38±11</td>
<td>159±4</td>
<td>52±4</td>
<td>20.7±1.6</td>
<td>123±30</td>
<td>67±31</td>
<td>667±151</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td>Group C</td>
<td>9</td>
<td>33±10</td>
<td>155±6</td>
<td>56±16</td>
<td>23.0±6.3</td>
<td>124±28</td>
<td>77±28</td>
<td>770±256</td>
<td>1.4±0.4</td>
</tr>
</tbody>
</table>

p-values: 0.34, 0.19, 0.74, 0.40, 0.85, 0.59, 0.41, 0.95

Wilcoxon signed rank test, and differences among the three groups on PODs 1 and 3 were compared using the Kruskal–Wallis test.

Results

All patients successfully underwent a transumbilical SILS procedure without open conversion. No complications associated with the block procedures were encountered. All patients satisfied the authors’ well-defined discharge criteria and were discharged to their homes on POD 5. There were no statistically significant differences with regard to age, body height and weight, body mass index, duration of anesthesia and surgery, and dose of remifentanil and propofol among the three groups (Table 1).

The frequency of intravenous administration of flurbiprofen was 1 (0–2), 1 (0–2), and 1 (0–2) on OPD and PODs 1–3 in Groups A, B, and C, respectively. The frequency of
oral administration of loxoprofen sodium hydrate was 1 (0–3), 1 (0–1), and 0 (0–2) on POD 1 ($\alpha = 0.27$), 0 (0–1), 0 (0–1), and 0 (0–1) on POD 2 ($\alpha = 0.76$), and 0 (0–2), 0 (0–1), and 0 (0–0) on POD 3 ($\alpha = 0.12$) in Groups A, B, and C, respectively.

The NRS scores were 1 (0–5), 2 (1–3), and 2 (0–3) on POD 1 and 1 (0–3), 1 (0–2), and 1 (0–2) on POD 3 in Groups A, B, and C, respectively. There were no significant differences in NRS scores among the three groups on POD 1 ($\alpha = 0.72$) and POD 3 ($\alpha = 0.60$). The scores on POD 3 were significantly lower than those on POD 1 in Groups B ($\alpha = 0.03$) and C ($\alpha = 0.02$), but not in Group A ($\alpha = 0.29$; Figure 3).

Table 2. — Distances walked in the 6-minute walk test (6MWT) (m).

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>$\alpha$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>494±63</td>
<td>468±57</td>
<td>448±74</td>
<td>0.33</td>
</tr>
<tr>
<td>POD 1</td>
<td>279±95</td>
<td>303±71</td>
<td>321±108</td>
<td>0.63</td>
</tr>
<tr>
<td>POD 3</td>
<td>394±72</td>
<td>399±76</td>
<td>421±80</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Discussion

The SILS procedure has recently come to be preferred as a more minimally invasive procedure compared with conventional laparoscopic surgery. However, this procedure is associated with substantial postoperative pain, probably because of extensive manipulation of the fascial wall beneath

Table 3. — Mean blood pressure and oxygen saturation before and after the 6MWT.

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>Group A POD 1</th>
<th>POD 3</th>
<th>Group B POD 1</th>
<th>POD 3</th>
<th>Group C POD 1</th>
<th>POD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg) before</td>
<td>115/75</td>
<td>114/76</td>
<td>115/76</td>
<td>118/75</td>
<td>116/75</td>
<td>111/72</td>
<td>118/77</td>
</tr>
<tr>
<td>after</td>
<td>121/79</td>
<td>113/74</td>
<td>117/78</td>
<td>122/76</td>
<td>128/81</td>
<td>112/78</td>
<td>125/80</td>
</tr>
<tr>
<td>$\alpha$ value</td>
<td></td>
<td>0.05</td>
<td>0.36</td>
<td>0.25</td>
<td>0.06</td>
<td>0.38</td>
<td>0.21</td>
</tr>
<tr>
<td>Oxygen saturation (%) before</td>
<td>98±1</td>
<td>96±1</td>
<td>97±1</td>
<td>98±1</td>
<td>98±1</td>
<td>98±1</td>
<td>98±1</td>
</tr>
<tr>
<td>after</td>
<td>98±1</td>
<td>97±1</td>
<td>98±1</td>
<td>98±1</td>
<td>98±1</td>
<td>98±1</td>
<td>98±1</td>
</tr>
<tr>
<td>$\alpha$ value</td>
<td></td>
<td>0.50</td>
<td>0.10</td>
<td>0.07</td>
<td>0.26</td>
<td>0.32</td>
<td>0.25</td>
</tr>
</tbody>
</table>
the umbilical skin incision. Ultrasound-guided TAP and RS blocks are now widely used as part of a multimodal analgesic regimen after abdominal surgery and are speculated to be effective in relieving umbilical pain after SILS procedures. To the present authors’ knowledge, no literature has reported the efficacy of regional anesthesia combined with general anesthesia, except for a case report by Matthes et al. [11] who reported that a 30-year-old woman who was breastfeeding her infant received TAP block with bupivacaine and successfully underwent SILS cholecystectomy without the need for intraoperative and postoperative opioids. The present randomized controlled trial systematically investigated the effects of preoperative TAP and/or RS blocks on the course of pain and mobility over three days after an SILS procedure.

No statistically significant difference was found in NRS scores among the three groups on PODs 1 and 3, whereas these had significantly decreased on POD 3 compared with those on POD 1 in the two groups who received the RS block (Groups B and C). This finding suggests that the RS block is more appropriate than the TAP block for gynecological SILS procedures, providing analgesia to the periumbilical tissues supplied by the branches of nerves T9–11.

The 6MWT was chosen for the evaluation of overall physical function in the acute postoperative period. This test is now the most extensively used among the many walking tests available, and it is currently recommended for use in both research and clinical settings [16]. When conducted safely following abdominal surgery, as in this study, the 6MWT is a useful objective tool to evaluate postoperative pain and measure clinical progress. The distance walked before surgery was comparable among the three groups, while on POD 1, the patients showed a marked decrease in all three groups. The distance walked recovered on POD 3 in the patients who had received the RS block alone (Group C), and it was not significantly different from that before surgery. In contrast, it remained significantly low on POD 3 in the patients who had received the TAP block alone or in combination with the RS block. Patients who received the RS block did not require postoperative analgesia on POD 3. These results suggest that the RS block alone is the most beneficial among the three block procedures in facilitating early mobilization after gynecological SILS procedures. Because this block relieves pain in the rectus abdominis muscle, which is used chiefly for standing and walking movements, it may have decreased movement-associated pain, minimizing the decrease in postoperative walking speed.

Enhanced Recovery after Surgery protocols aim at decreasing the surgical stress response and optimizing recovery, thus decreasing the length of hospital stay [17]. Bed rest after surgery is undesirable because it impairs pulmonary function and tissue oxygenation and predisposes the patient to pulmonary complications. To avoid this, it is important to mobilize patients as soon as possible after surgery. Therefore, recovery of the distance walked in patients who receive the RS block may lead to a decrease in postoperative complications, although the length of the postoperative course was similar among the three groups in this study.

This study had some limitations. First, sensory block observation was not included in the study protocol. Second, the sample size of each group was small. It cannot be readily explained why the RS block using ropivacaine was effective in decreasing pain until POD 3. Little is known about the pharmacodynamics and pre-emptive analgesic effects of ropivacaine used in TAP and RS blocks.

**Conclusion**

The RS block using the same total dose of ropivacaine proved to be more effective for postoperative pain control and general recovery compared with the TAP block alone and in combination with the RS block in the early period after gynecological SILS procedures. Early pain relief may enhance postoperative recovery and contribute to early rehabilitation. Further investigation is necessary to establish the method of regional analgesia that is most appropriate for SILS procedures, which are now enjoying more widespread use in laparoscopic surgery.

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Histologic changes caused by nonabsorbable sutures after ovarian suspension

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Summary

Purpose: To evaluate intraovarian histologic changes caused by polypropylene and silk sutures that commonly are used in ovarian suspension. Materials and Methods: Twenty-four female rats were randomly allocated to three study groups: a sham group receiving no ovarian suspension; the other two groups that had right ovarian suspension with polypropylene and silk sutures. At 90 days after surgery, the histologic changes and ovarian weight reduction in the suspended ovaries and severity of pelvic adhesions were evaluated. Results: There were no differences between study groups in focal inflammation, cystic structures, or vascularity. Adhesion severity and ovarian weight reduction in suspended ovaries and cysts around the suspended ovary were significantly greater in the silk than sham group. The frequency of hematoma within the suspended ovary was significantly greater in the polypropylene than sham group. Conclusions: Polypropylene suture caused less adhesion severity or ovarian weight reduction than silk suture. This suggests that polypropylene suture may be the better suture for ovarian suspension procedures.

Key words: Inflammation; Adhesions; Cysts; Ovary; Polypropylene; Silk.

Introduction

During the reproductive years, the ovaries are very sensitive to the harmful effects of pelvic irradiation. The frequency of ovarian failure after radiation therapy is from 12% to 66% [1, 2]. Therefore the ovaries should be protected from the harmful effects of pelvic radiation therapy.

Ovarian suspension is a surgical procedure in which the ovary is transposed to a different location and fixed using transparenchymal sutures. The procedure is done to protect the ovaries from the harmful effects of radiation during pelvic radiation therapy or to prevent recurrent ovarian torsion. Nonabsorbable suture materials such as polypropylene or silk may be used in ovarian suspension procedures [3-7]. The ovaries may be detached from the uterus and suspended with their blood supply at a region outside the pelvis, usually the lateral paracolic gutter above the pelvis. Ovarian suspension performed before radiation therapy may preserve ovarian function in 16% to 90% patients [8, 9]. Failure of ovarian suspension to preserve ovarian function may be associated with age of the patient, not shielding the ovaries during radiation therapy, scattered radiation, adjuvant chemotherapy, and decreased blood supply to the suspended ovary. In addition, foreign bodies such as sutures placed in ovarian tissue during suspension may impair ovarian function.

Oophoropexy, also known as ovariopexy, is performed to prevent recurrent ovarian torsion. This procedure is similar to ovarian suspension. The surgical technique includes suturing the ovary with a transparenchymal suture to the pelvic side wall or the round ligament and plicating the utero-ovarian ligament [7, 10].

Previous studies have evaluated histologic changes or paraovarian adhesion formation during ovarian reconstruction using various suture materials [11-13]. However, there is limited information available about the effects of nonabsorbable suture materials on the ovary, such as inflammatory changes in the ovary or pelvic adhesions that occur after ovarian suspension. This information may be important because ovarian parenchymal changes may have detrimental effects on ovarian function and fertility.

The purpose of this experimental study was to evaluate intraovarian histologic changes caused by polypropylene and silk sutures that commonly are used in ovarian fixation procedures.

Materials and Methods

Animals

This experimental study was performed with 24 female Sprague Dawley rats that were randomly allocated into three study groups (eight rats per group): a sham group receiving no ovarian suspension, a group that had right ovarian suspension with polypropylene sutures, and a group that had right ovarian suspension with silk sutures. The study was approved by the animal experimentation local ethics board of Hacettepe University.

Procedure

Intraperitoneal anesthesia was induced with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg). In
all rats, a two-cm abdominal incision was made, both ovaries were identified, and left ovariectomy was performed. In the rats in the polypropylene and silk groups, right ovarian suspension was performed; the connection between the right ovary and the uterine horn was cut, and the ovary was fixed with the vascular pedicle to the peritoneum in the right paracolic region superiorly using transparent transparanchymal sutures (polypropylene 5-0 or silk 5-0). The excised left ovaries were weighed and the abdomen was closed with silk 3-0 suture in two layers.

After surgery, the animals were housed in a temperature- and humidity-controlled environment at 22°C on an alternating cycle of light (12 hours) and darkness (12 hours). The rats were fed typical laboratory food and tap water ad libitum. At 90 days after the initial surgery, the same type of anesthesia was induced, and similar laparotomy was performed. The severity of pelvic adhesions was graded in each animal with a standardized scale and a corresponding score was obtained for each rat in a blinded manner; adhesion severity was described and scored as absent adhesions (0 points); thin, filmy adhesions (1 point); definite localized adhesions (2 points); dense multiple visceral adhesions (3 points); or dense adhesions extending from the abdominal wall to visceral organs (4 points) [14]. Hematoma within the suspended ovaries and cysts around the suspended ovaries were recorded. The suspended ovaries were removed and weighed. The weight difference of ovaries after 90 days was calculated between control (left) and suspended (right) ovaries in each rat.

**Histology**

The histologic changes in the suspended or sham ovaries were investigated. Fresh tissue samples were rapidly fixed in 10% phosphate buffered formalin, dehydrated through graded alcohol solutions, and processed for routine light microscopy. All specimens were embedded in paraffin blocks, and five-µm sections were cut and stained with hematoxylin-eosin. Sections were examined using a microscope and photographed. A single histologist evaluated the histologic changes in a blinded manner. The histologic examination evaluated superficial inflammatory changes, cystic structures, vascularity, and follicular maturation.

Superficial inflammatory changes adjacent to the suture were graded as none, mild, moderate, and severe and were assigned numerical values of 0 to 3 as previously described [15]. Mild inflammatory changes were defined as a focal inflammatory infiltrate involving < 25% ovary with adjacent normal ovarian structure. Moderate inflammatory changes were defined as 25% to 75% involvement of the ovary. Severe inflammatory changes were defined as > 75% involvement of the ovary.

Cystic structures (dilation of lymphatic vessels containing exudate without red blood cells) were graded based on the number of cystic structures in an area with ten-fold magnification (grade 1 had one to ten cystic structures; grade 2 had 11 to 20 cystic structures; and grade 3 had > 20 cystic structures). Vascularity was scored similar to cystic structures. The grading systems were assigned numerical values for statistical analysis. In addition, primordial and maturing follicles (primary, secondary, and antral follicles) were evaluated for each rat.

**Statistical analysis**

Data analysis was performed with statistical software (SPSS version 20). The scores of focal inflammation, cystic structures, vascularity, and adhesion severity were not normally distributed; the non-parametric Kruskal-Wallis test was used to compare these variables between the groups. Pairwise differences were evaluated with Mann-Whitney test with Bonferroni adjustment for multiple comparisons. Weight differences between the ovaries were normally distributed and were compared with one-way analysis of variance. Pairwise post hoc analysis was performed with Tukey test. The $\chi^2$ test (chi-square test) or Fisher exact test was used to compare proportions of hematoma within, and cysts around, suspended ovaries in different groups. Bonferroni adjustment was applied for multiple comparisons. Statistical significance was defined by $p \leq 0.05$.

### Results

At 90 days after ovarian suspension, there were no differences between study groups in focal inflammation, cystic structures, or vascularity (Table 1). Inflammation, cystic structures, and vascularity in the suspended ovary typically were grade 1 or 2 in all rats (Figure 1). In the silk group, only one rat presented with grade 3 cystic structures and vascularity in the suspended ovary (Figures 2, 3).

Adhesion severity was significantly greater in the silk than sham group (Table 1). Ovarian weight reduction in suspended ovaries was significantly greater in the silk than sham group, and there was no difference in weight reduction between the polypropylene and sham group (Table 1).

The frequency of hematoma within the suspended ovary was significantly greater in the polypropylene than sham group, and cysts around the suspended ovary were significantly greater in the silk than sham group (Table 1). There was one abscess (2 × 2 cm) noted around the suspended ovary in the polypropylene group.

In all study groups, each animal had normal primordial and maturing follicles (primary, secondary, and antral follicles) in the suspended or sham ovary (Figures 4, 5).

### Table 1. — Histologic changes associated with ovarian suspension in rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Polypropylene</th>
<th>Silk</th>
<th>$p$ ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Focal inflammation</td>
<td>1.4 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.6 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Cystic structures</td>
<td>1.3 ± 0.5</td>
<td>1.6 ± 0.5</td>
<td>1.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Vascularity</td>
<td>1.5 ± 0.5</td>
<td>1.4 ± 0.5</td>
<td>1.5 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Adhesion severity</td>
<td>0.4 ± 0.5</td>
<td>0.5 ± 0.9</td>
<td>2 ± 1</td>
<td>0.01*</td>
</tr>
<tr>
<td>Weight difference (mg)*</td>
<td>2 ± 5</td>
<td>-5 ± 8</td>
<td>-9 ± 5</td>
<td>0.005*</td>
</tr>
<tr>
<td>Hematoma within suspended ovary</td>
<td>0 (0)</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>0.002b</td>
</tr>
<tr>
<td>Cyst around suspended ovary</td>
<td>0 (0)</td>
<td>1 (13)</td>
<td>4 (50)</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

* N = 24 rats. Results at 90 days after sham surgery or ovarian suspension with polypropylene or silk transparent suture. Data reported as mean ± SD or number (%).

*NS, not significant ($p > 0.05$).

* With multiple comparisons and Bonferroni adjustment, differences were significant between sham vs silk groups for adhesion severity, sham vs polypropylene groups for hematoma within suspended ovary and sham vs silk groups for cyst around suspended ovary.

* Weight difference between control (left) and suspended (right) ovaries.

* With post hoc test, difference between sham and silk groups was significant.
Discussion

This experimental study showed that suspension of the ovary with transparenchymal polypropylene or silk sutures was associated with no significant histologic changes in focal inflammation, cystic structures, or vascularity within the suspended ovaries compared with sham ovaries (Table 1). Severity of adhesions in the pelvis, weight reduction in the suspended ovary, and frequency of cysts around the suspended ovary were significantly greater in the silk than sham group (Table 1). Both polypropylene and silk sutures had no effect on primordial and maturing follicles (Figure 4).

Surgical procedures on ovary such as ovarian suspension, ovarian reconstruction, or cystectomy are performed with various types of sutures that are placed inside the ovary. However, very limited data are available about the effects of suture materials within ovarian tissue. Therefore, comparison between the present and previous studies is limited.
In a previous experimental study that compared ovarian reconstruction using rapid acting human fibrin glue, polyglactin absorbable suture, and no suture, results with the different materials were similar in adhesions, ovarian size, presence of cysts, and histologic scores [13]. In contrast, the present study showed that nonabsorbable suture material caused changes in adhesions, ovarian weight, hematoma, and cysts (Table 1). Atrophy or weight reduction in the suspended ovary, greater in the silk than sham group, may have been caused by vascular compromise in the suspended ovary caused by the suture. Edema may develop in response to suture materials and may potentially mask the actual weight reduction in the suspended ovary, and this may explain why no significant weight reduction was demonstrated with polypropylene suture (Table 1).

Previous studies showed that ovarian suspension may cause the development of cystic structures in 23% to 24% ovaries [8, 16]. Although the authors observed no intraovarian cysts in suspended ovaries, cystic structures around the suspended ovary with clear serous fluid were detected with propylene and silk sutures but not in the sham group (Table 1). These cystic structures around the suspended ovaries had the appearance of peritoneal inclusion cysts, which may be caused by peritoneal adhesions after ovarian suspension [17]. Therefore, the differential diagnosis of postoperative ovarian cysts in suspended ovaries may include peritoneal inclusion cysts.

Although good functional outcomes have been reported in ovaries after suspension [18], the effects of suture materials on developing or mature follicles within the suspended ovary have not been evaluated in detail. In five young girls with radiosensitive brain tumors who had laparoscopic ovarian suspension with polypropylene suture before radiation therapy, ovarian biopsy after radiation therapy showed > ten germinal follicles per high power field [19]. Therefore, ovarian suspension may enable preservation of ovarian germinal follicles [19]. In the present study, primordial and maturing follicles also were not affected by suture material. The absence of harmful effects of suture materials on ovarian follicles may explain, in part, the frequent preservation of ovarian function after suspension.

Ovarian suspension may cause postoperative adhesions and interfere with the anatomic relation between pelvic organs. In two previously reported patients who had laparoscopic ovarian suspension with polypropylene suture before radiotherapy, no adhesions were noted on laparoscopy at three months after radiation therapy [20]. This is consistent with the present results that showed similar adhesion severity in the pelvis between polypropylene and sham groups (Table 1). However, the silk group had significantly greater adhesion severity than the sham group (Table 1). Therefore, polypropylene may be better than silk suture in minimizing the development of pelvic adhesions after ovarian suspension.

Limitations of the present study included the possibility that the animal model may not necessarily mimic typical ovarian suspension in human clinical practice. In addition, the short study duration (90 days) precluded the determination of long-term histologic and functional outcomes. Nevertheless, the study improves the available information about the effects of transparenchymal suture materials within ovaries after suspension and may serve as a model for the evaluation of ovarian suspension in humans.

Conclusions

This study focused on ovarian tissue reactions to two different types of suture materials in an animal model and provided information about tissue reactions at 90 days after suture placement. Transparenchymal polypropylene and silk sutures caused no significant histologic changes in focal inflammation, cystic structures, or vascularity between the suspended and sham ovaries. Polypropylene suture caused less adhesion severity or ovarian weight reduction than silk suture. This suggests that polypropylene suture may be the better suture for ovarian suspension procedures. However, further study is necessary to determine the long-term histopathologic and functional changes associated with these suture materials in the ovary.

Competing interests: the authors declare that they have no competing interests.

Authors’ contributions: SH participated in the design of the study and in drafting the manuscript and carried out experiments. LKS carried out histologic assessments and helped to draft the manuscript. FFK carried out histologic assessments and helped to draft the manuscript DH participated in the study design and coordination and helped to draft the manuscript. HT participated in the study design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

References

Histologic changes caused by nonabsorbable sutures after ovarian suspension

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Exaggerated placental site/placental site trophoblastic tumor: an underestimated risk factor for emergency peripartum hysterectomy

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Departments of Obstetrics & Gynecology¹ and Pathology², Hospital Universitario “Marqués de Valdecilla”, Universidad de Cantabria, Santander (Spain)

Summary
Objective: To assess the indications and possible underlying causes of emergency peripartum hysterectomy (EPH) at the present hospital during the 2001-2011 period. Materials and Methods: A revision of the charts and pathology reports corresponding to 42,728 par- turnents. Results: During the study period, 25 peripartum hysterectomies were performed (0.61/1.000), of which 23 were EPHs (0.54/1.000) and two were planned cesarean hysterectomies. The indication for EPH was acute postpartum hemorrhage in 22 of 23 instances (95.7%). Roughly two-thirds of the operated uteri (16/25, 64%) showed placental site anomalies, half corresponding to different degrees of placental accretism and half to anomalies derived from the implantation site intermediate trophoblast. In five cases (31%), the anomaly was an exaggerated placental site and three cases corresponded to placental site trophoblastic tumors. Of the 16 cases showing placental site anomalies, ten (62.5%) were associated with one or more previous cesarean sections. Conclusions: Roughly one-third of EPHs performed at the present center during the last ten years were associated with placental site anomalies originating in the implantation site intermediate trophoblast (exaggerated placental site and placental site trophoblastic tumor). This association has not been described before, and should be taken into consideration when facing acute peripartum hemorrhage predisposing to EPH.

Key words: Emergency peripartum hysterectomy; Placental site.

Introduction
In modern obstetric practice of developed countries, unmanageable postpartum hemorrhage or other conditions requiring emergency peripartum hysterectomy (EPH) are rather exceptional. However, during the last two decades there has been an apparent rise in the frequency of EPH precisely in these countries [1]. Two factors preeminently associated with the incidence of EPH in modern series from the developed world are placental site anomalies, specifically different degrees of accretism, and previous cesarean section, which are themselves interrelated. Since cesarean section rates in developed countries are nowadays significantly higher than in the immediate past, the corollary is that the rising cesarean section rate has carried with it an upsurge of EPH as an unwanted side-effect [1, 2]. In comparison, classical risk factors such as uterine atony, uterine rupture, placenta previa, and sepsis are still the major risk factors for EPH in less developed countries, even in those with the most up-to-date technical facilities [3-5].

In our medium there is a prevalent feeling, especially among midwives, that the incidence of EPH is not only rising, but getting out of control, although the objective figures tell us that the real incidence has remained stable over the years, and well within the boundaries of international standards. However, it may well be that the pattern of related causes has significantly shifted during later times, as has happened in other countries, giving the false impression that there is an unjustified explosion of EPHs, because formerly they were almost exclusively associated with uncontrollable uterine atony, something that has virtually disappeared from modern obstetric wards. Therefore, in the absence of the major classical cause of EPH, it appears inexplicable that there might be a relative increase thereof. The present authors have carried out the present study in order to analyze the exact situation regarding EPH and its indications in the present hospital, which is a tertiary-care, university-based teaching center.

Materials and Methods
The authors revised the charts of mothers submitted to EPH at Hospital Universitario “Marques de Valdecilla”, Cantabria University, Santander, Spain, between January 1st, 2001 and December 31st, 2011. During this period, there were 23 EPHs and two planned cesarean hysterectomies among 42,728 deliveries, for an overall incidence of 0.61 per 1,000 deliveries, and an adjusted incidence specifically for EPH of 0.54. The cesarean section rate remained stable around 20% (range: 18.1 – 24.9%) during this time span, with a slight decline during 2001 and 2002 and during the last two years of the study.

The clinical variables studied in relationship with EPH were age, parity, previous obstetric history, present obstetric history, kind of delivery, placental site anomalies, uterine anomalies, time interval between the onset of uterine hemorrhage and hysterectomy, medical interventions, indication for hysterectomy and pathological status of the surgical specimens. All excised...
uteri and placentae were examined or revised by the same pathologist (IGR), who is the consultant for Obstetric and Gynecologic Pathology at the present hospital.

Results

In all, 25 hysterectomies (14 total, 11 subtotal) were performed during the study period, of which 23 were EPHs and two planned cesarean hysterectomies. Of the corresponding 25 mothers, 18 (72%) delivered by cesarean section and seven vaginally. If compared to the general study population, this difference in the incidence of cesarean section is statistically significant (Chi-square test, \( p < 0.0001 \)). The indication for EPH was acute postpartum hemorrhage in 22/23 instances (95.7%). The only exception was a patient who developed a uterine necrosis secondary to an episode of acute appendicitis on the eighth day of puerperium. Out of the 22 patients with acute postpartum hemorrhage requiring EPH, 11 (50%) additionally developed disseminated intravascular coagulation. The onset of acute postpartum hemorrhage took place within the first hour postpartum in ten cases and between one and eight hours postpartum in 12 cases.

Two patients, finally, underwent planned cesarean hysterectomies, because of placental insertion anomalies (placenta increta and placenta percreta), correctly suspected by ultrasound and diagnosed by magnetic resonance imaging during their gestation.

Patient features associated with the indication of EPH were, in descending order: age above 35 years (17/25, 68%), one or more previous cesarean sections (11/25, 44%), and placenta previa (8/25, 32%).

The pathological examination of the operative specimens disclosed that roughly two-thirds of the uteri (16/25, 64%) showed placental site anomalies. Half of them corresponded to different degrees of accretism (five placenta accreta, two placenta increta, and one placenta percreta). In five cases (31%), the anomaly consisted of a seldom diagnosed entity in this context, exaggerated placental site (Figure 1). An additional case was a combination of exaggerated placental site and placenta accreta, and was ascribed to the just mentioned group of placenta accreta. The remaining three cases were placental site trophoblastic tumors, also a relatively rare condition. Of these 16 cases showing placental site anomalies, finally, ten (62.5%) were associated with one or more previous cesarean sections. All pathological diagnoses are summarised in Table 1.

Discussion

The reported incidence of peripartum hysterectomy in some of the most advanced obstetric services of developed countries ranges between 0.4 and 0.85 per 1,000 deliveries [1, 2], which is approximately ten times lower than the figures reported for developing countries with comparable hospital facilities [3-5]. Our incidence of 0.54 per 1,000 lies well within the range for developed countries, of which our own forms part. Furthermore, the present authors registered also the same rate of main predisposing or associated causes reported in modern maternities, i.e., abnormal placentation causing postpartum hemorrhage, associated in turn to a history of previous uterine (cesarean) scar [1, 2]. In fact, roughly two-thirds of the present patients presented with anomalies of the placental site, and again roughly two-thirds of these arose on uteri having undergone one or more previous cesarean sections. One unexpected finding in the present series was that, of the placentation anomalies found, half of them, eight in all, typically corresponded to different degrees of macroscopically evident placental accretism, whereas, out of the other eight cases, five corresponded to exaggerated placentation, and three to placental site trophoblastic tumors. To the present authors’ knowledge, the latter two entities have not been associated with an increased incidence of EPH before. It is interesting to note that, although both placental site trophoblastic tumor and exaggerated placental site derive from the same cell family, i.e., implantation site intermediate trophoblast [6], they are not genetically related [7], and therefore, one cannot be considered a precursor of the other. Nevertheless, it can be speculated that some kind

<table>
<thead>
<tr>
<th>Pathological findings</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental anomalies</td>
<td>16/25</td>
<td>64.0%</td>
</tr>
<tr>
<td>Placenta accreta</td>
<td>8/16</td>
<td>50.0%</td>
</tr>
<tr>
<td>Exaggerated placental site</td>
<td>5/16</td>
<td>31.3%</td>
</tr>
<tr>
<td>Placental site tumour</td>
<td>3/16</td>
<td>18.7%</td>
</tr>
<tr>
<td>Uterine necrosis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Amniotic fluid embolism</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Abruptio placentae</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Normal puerperal uterus</td>
<td>6</td>
<td>24.0%</td>
</tr>
</tbody>
</table>

Table 1. — *Emergency peripartum hysterectomies 2001-2011. Pathological findings (n = 25).*
of alteration involving implantation site intermediate trophoblast, the implantation site itself or, more likely, the interaction between both, predisposes the uterus to acute peripartum hemorrhage, which in its turn leads to EPH.

In conclusion, roughly one-third of EPHs performed at the present center during the last ten years were associated with placental site anomalies originating in the implantation site intermediate trophoblast (exaggerated placental site and placental site trophoblastic tumor). This association has not been described before, and should be taken into consideration when facing acute peripartum hemorrhage predisposing to EPH.

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Treatment of tubal pregnancy using comprehensive interventional methods

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Summary
Objective: To investigate the efficacy of combined interventional methods in treatment of tubal pregnancy. Materials and Methods: One hundred sixty-two patients with tubal pregnancy were enrolled in this study. In all patients, the feeding uterine artery at affected side was perfused with methotrexate (MTX), followed by occlusion using gelatin sponge. Nineteen patients were also treated by perfusion of MTX in ovarian artery at affected side which partially participated in blood supply. Seven patients received direct puncture perfusion of MTX under B ultrasound guidance. Four cases received perfusion of MTX through fallopian tube. After surgery, the serum beta-human chorionic gonadotropin (β-HCG) level was regularly detected, and B ultrasound was used to monitor the pelvic mass change. For 33 patients with fertility requirement, hysterosalpingography (HSG) was conducted after menstruation restoration. Results: Tubal pregnancy was terminated in 160 patients (98.76%), with inefficacy in two patients (1.23%) who were treated by surgery. HSG showed tubal patency in 27 patients. Tubal obstruction was found in the other six patients. After recanalization, three cases were unobstructed, with obstruction in other three cases. Fifteen patients achieved intrauterine pregnancy after six to 17 months from surgery. Conclusions: Comprehensive interventional treatment can prevent internal bleeding caused by failure of many conservative treatments, improve the indication and success rate of treatment, and preserve the complete fallopian tube.

Key words: Tubal pregnancy; Interventional treatment; Efficacy.

Introduction
Tubal pregnancy is a common acute abdomen in obstetrics and gynecology department and acute serious hemorrhage caused by the rupture or abortion from tubal pregnancy can endanger the life of the pregnant woman. There are many causes of tubal pregnancy: inflammation and surgery are the main factors, in addition, chlamydia trachomatis infection [1], abnormal change of estrogen 17β-estradiol level [2], and low level expression of adrenomedul (ADM) [3] are closely related to pathogenesis of tubal pregnancy. Traditional treatment methods are surgery or expectant treatment, but fallopian tube ablation of most affected side can influence reproductive capacity of the patient; expectant treatment fails due to bleeding in most cases. Constant research on how to treat tubal pregnancy safely and effectively and retain the fertility of the patient as much as possible is therefore important. Interventional therapy, which has small trauma, is perfect and accurate and has low complication, and has been introduced in clinical practice in obstetrics and gynecology [4].

The goal of this study was to explore how to use synthetic interventional therapy to treat tubal pregnancy. From 1997 to this day, the authors selected 162 cases with tubal pregnancy diagnosed in their hospital to perform synthetic interventional therapy, such as perfusion and embolism in uterine artery and/or perfusion and chemotherapy in fallopian tube.

Materials and Methods
Clinical data
From October 1997 to the present, the authors selected 162 patients with tubal pregnancy in their hospital that were finally diagnosed synthetically by serum β-HCG , type-B ultrasound, and clinical symptoms and were willing to accept interventional therapy. Their age range was 20-42 years, mean age was 32.6 years, and there were 99 cases with tubal pregnancy in the right side and 63 cases in left side. There were 126 unmarried patients, 138 who did not bear children, and 24 who had an intrauterine device (IUD). Among the total, there were seven cases that had repeated tubal pregnancy, three cases that had mistakenly performed induced abortion, one case that had mistakenly performed drug abortion, and one case that had combined rheumatic heart disease and mitral valve stenosis. There were 25 cases combined with peritoneal cavity bleeding, but with stable vital signs. Suppressed menstruation time was 27-69 days; white cell count in each was more than 3.5×10⁹/l, all patients had different degrees of abdominal pain, there was maximum diameter line of 8.0 × 4.9 cm for pelvic cavity mass, and maximum diameter line of 4.1 × 2.5 cm for the blastocyst determined by type-B ultrasound. There were 27 cases with hydrops in pelvic cavity and the widest anteroposterior diameter in the hydrops in pelvic cavity was 3.8 cm. The authors performed culdocentesis in 16 patients, extracting 1.5 - 5 ml of unclotted blood; among the total, there was one case combined with 1.8 ml hydrops in liver-kidney interspace and 0.3 ml hydrops in spleen-kidney interspace at the same time, there was one case combined with 3.9 ml seropertoneum, and beta-human chorionic gonadotropin (β-HCG) value in serum was 67.8 ~ 491.61 IU/l (normal value < 15 IU/l). This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Tianyou Hospital Affiliated to Wuhan University of Science and Technology. Written informed consent was obtained from all participants.

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Method

The authors adopted the Seldinger technique through femoral artery, and inserted 5F super-smooth Cobra catheter via the uterine artery in affected side, and performed an arteriography to confirm abnormal staining in the area of fallopian tubes; this group of cases were divided into three types of vessels signs according to the manifestation of the angiography. They instilled MTX + physiological saline at three ml/min with constant pressure pump, type I-II patients used 60~100mg MTX, and type III patients used 100~120mg MTX. After the completion of drug instillation, the authors used gelfoam particulate for embolization of uterine artery and performed an arteriography at the opposite side for 30 patients randomly; the result did not show any change, similar to affected side by comparison. There were six cases that required instillation chemotherapy as well, who showed thickening arteria ovarica because of contrast media reflux when performing arteriography in uterine artery in affected side. There was one case in which the embolism of ramus splanchnicus of internal iliac artery was performed, because the catheter could not be inserted in the uterine artery as the patient resolutely rejected operative treatment. There was a fetal heart beat after two days as detected by ultrasonic color Doppler, therefore 50 mg MTX were instilled to terminate the pregnancy with cannula from the cervix to the opening of fallopian tubes in affected side.

Treatment after the operation and monitoring indexes

The authors used three mg of formyltetrahydrofolate (CF) intramuscularly 12 hours after the operation, once every four hours for four times total and also prescribed anti-inflammatory hydra-
At follicular phase, four to five ml fasting venous blood was taken from the patients, serum was separated, and stored at -20°C. E2, LH, and FSH kits were utilized according to the manufacturer’s instructions. A radioimmunoassay and KH-6020 γ immunity counter were also utilized.

Statistical analysis
SPSS 17.0 statistics software was used and each parameter was expressed as ± s; t-test was used for the comparison between pre-therapy and post-treatment. There was statistical significance between the difference if \( p < 0.05 \).

Results

Angiographic image of tubal pregnancy
Tubal pregnancy has extensively altered vascularization: angiography showed that the gestational sac originated from tubal branches of the uterine and ovarian arteries that can contribute to the sac’s vascularization, and in few cases also from anastomotic branches. They were divided into three types according to angiographic signs in 162 patients and according to the comparison of the arteriography of uterine artery in opposite side in 30 patients. Type I: no obvious positive signs in 15 cases (9.25%) (Figure 3); Type II: tubal branches from the uterine artery at pregnancy side were thickened, with no blood-supply vessels to gestational sac, and there were stained filaments in parenchymal phase, and complete gestational sac was not visible in 26 cases (16.05%) (Figure 4); Type III: tubal branches from uterine artery at pregnancy side was clearly thickened, arteriole branches originated from the tubal branches that supplied the gestational sac, there were distinctive vessel signs of gestational sac in advanced stage of artery and parenchyma phase; a filament was clearly stained, the shape was round, the border was irregular, the staining was even or uneven,
there was no significant difference between six month post-treatment and pre-therapy (p = 0.942), LH (p = 0.975), FSH (p = 0.997). There was no significant difference between 12 month post-treatment and pre-therapy with respect to the change of E2 (p = 0.942), LH (p = 0.999), and FSH (p = 0.987) (Table 1).  

Discussion  
Tubal pregnancy is a commonly encountered disease in obstetrics and gynecology department; more than 98% of ectopic pregnancy occur in fallopian tubes [5, 6], especially in the ampulla. Once pregnancy ruptures, bleeding amount is often extensive, shock can appear in short time, which can cause infertility and can even endanger the patient’s life. It is the main causes of death of pregnant woman during gestation, because ectopic pregnancy cannot be diagnosed and treated in time. Therefore, it is very important to search for a method which can integrate diagnosis and treatment, cause smaller trauma, and that can offer a reliable therapeutic effect and maintain reproductive organs intact.

In this study, 162 patient angiogram results were grouped and selective uterine artery cannulation chemotheraphy and embolotherapy were performed, combined with MTX injection with traditional techniques either per abdomen or vagina with ultrasound guidance at the same time. Out of 162 patients, 160 patients were cured. In patients who had fertility requirements, 33 patients underwent HSG postoperatively and the results showed that there were 31 patients with unobstructed fallopian tubes; among the total, there were 15 patients that achieved an intrauterine pregnancy within 17 months postoperatively. There was no obvious change between six and 12 postoperatively with respect to preoperative E2, LH, and FSH.

This experiment indicates that this method has higher achievement ratio, fewer complications, and less effect on normal fertility as well as on sex hormone levels in treating tubal pregnancy.

Tubal pregnancy was divided into operative and non-operative treatment. Non-operative treatment mainly included expectant treatment and medicinal treatment. Expectant treatment was only suitable for a part of low-risk tubal pregnancies and the effective difference was significant. Emma et al. [7] found that achievement ratio was between 48%-100% due to the difference of selected criteria. Medicinal treatment can avoid surgery and postoperative complications but has strict indications, and is only suitable for ectopic pregnancy in early stage and patients who have no prior pelvic inflammation. Hossam et al. [8] performed the comparison between single dose and double dose MTX and the results showed an achievement ratio of 72% and 79%, respectively. Initiated fallopian tube rupture was found in 3.8% and 2.5%, respectively.

MTX treatment can include some risks such as allergy, embryopathy, and so on [9]. Compared with non-operative treatment, achievement ratio of synthetic interventional therapy occurs in 98.77%; there are only 1.23% of patients who have fallopian tube rupture, and side-effects are due to local application drugs. Treatments are mainly divided into open abdominal surgery, peritoneoscopic surgery, and interventional therapy. Many open abdominal surgeries are radical operations which resect fallopian tubes in affected

Table 1. — Comparison of sex hormone levels between pre-therapy and post-treatment (x ± s).

<table>
<thead>
<tr>
<th></th>
<th>E₂ (pg/ml)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-therapy</td>
<td>68.18 ± 11.53</td>
<td>6.23 ± 1.35</td>
<td>4.78 ± 1.30</td>
</tr>
<tr>
<td>Six months after treatment</td>
<td>64.34 ± 12.61</td>
<td>6.16 ± 1.39</td>
<td>4.80 ± 1.34</td>
</tr>
<tr>
<td>Twelve months after treatment</td>
<td>67.09 ± 11.25</td>
<td>6.25 ± 1.42</td>
<td>4.72 ± 1.33</td>
</tr>
</tbody>
</table>
side. Many peritoneoscopic operations are conservative embryo removal techniques. A report [10] abroad showed that the application of conservative surgery with the peritoneoscope is more common than open surgical procedures, which has become main therapeutic method for tubal pregnancy.

Fallopian tubes in affected side is resected or conservative surgery treatment with peritoneoscope and can leave a scar in fallopian tubes, which not only brings higher injury to the body, but can be easily complicated with bleeding, infection, ureteral injury, intestinal obstruction, fallopian tubes stenosis and so on, which also have an effect on fertility. Shavit et al. [11] reported that ectopic pregnancy can recur after salpingectomy. Although single foramen peritoneoscope has more advantages than traditional peritoneoscope treatment, it still includes complication such as the bleeding, incisional hernia, and so on [12].

To achieve acute ischemia and necrosis of ectopic nidation embryo, the present authors adopted metaphase non-permanent gel foam particulates to embolize the uterine artery. It not only made internal hemorrhage decrease or stop, but also avoided having to open the abdomen while it preserved integral fertility of the patients. Blockage of supply blood is an effective therapeutic tool for the hemorrhage after cesarean section, which has been described since 1952; achievement ratio of treating hemorrhaging patients after childbirth and cesarean section by ligating uterine artery is up to 90% [13]. In 1970s, Farrer-Brown et al. used uterine artery embolism to treat postpartum hemorrhage [14].

According to arteriography change of uterine artery of 162 patients with tubal pregnancy in affected side and the comparison with arteriography of 30 cases among the total in normal side randomly, the present authors found that the uterine artery sends out slight tubal branches from fundus of uterus to supply fallopian tubes, which is the main blood supply vessel for tubal pregnancy and has anastomotic branches with the uterine artery. When tubal pregnancy occurs, because fertilized ovum imbeds in fallopian tube lumen, trophoderm splits and increases, the chorion grows and filament vessels form; when the arteriography of uterine artery was performed, contrast media enters into abundant filament vessel, it can be seen there are agglomerate or lamella staining of floss vessel in the area of fallopian tubes besides the uterus in parenchymal phase of the angiography, which shows characteristic vessel sign when performing arteriography. It can provide reliable evidence for tubal pregnancy to further clear diagnose and selectivity uterine artery cannulation.

Interventional therapy is gradually being accepted and adopted as a newly emerging therapeutic tool. For treating fallopian tube diseases, the basic operation is selective fallopian tube cannulation. Platia and Krudy [15] are the first to report interventional therapy of fallopian tubes. Traditional interventional therapy is mainly introduced by ultrasound per abdomen, per vaginal, X-rays, uteroscope, and so on, which uses selective fallopian tube cannulation and injects certain drugs such as MTX and mifepristone on affected side of fallopian tubes to locally treat tubal pregnancy. However traditional interventional therapy has some risks such as inflammatory blockage and perforating of fallopian tubes, which are important reasons that cause infertility [16]. The present data showed that the recovery rate for synthetic interventional therapy was 98.76%. Synthetic treatments reports that incidence rate of general side-effects caused by MTX is 21% and that of topical drug administration is 2%. Topical drug administration aim is to inhibit or kill filament nutrient cell, cause ectopic embryo growth to pause, necrotize, and disappear, and provoke lesser injury to fallopian tube tissue as much as possible, and protect fallopian tubes from obstruction. In the present study, the authors selected different treatment protocols according to the different angiographic results, ensured that the drug arrived to fallopian pregnancy site directly and quickly, decreased drug collateral side-effect, while improving the therapeutic effect at the same time.

Shalev et al. reported that less than eight weeks of gestational is the optimal time for topical drug infusion therapy [17]. The present authors previously thought that drug filling therapeutic effect is certain if embryonic sac diameter was less than 3.0 cm. There were 25 patients suffering from tubal pregnancy and then they developed abortion or rupture, 14 patients whose blastocyst diameters were more than 3.0 cm, and 22 patients whose gestation weeks were more than eight, therefore the authors increased MTX dose moderately for patients with type III angiographic change: gestational sac in this type may implant in mucous membrane deep layer or lamina muscularis, blood supply is abundant, and filament tissue vitality is strong. This difference may provide evidence for confirming the doses in chemotherapeutics.

There are several factors influencing the effect in applying synthetic interventional therapy: the first factor is to confirm the indication of the operation, the second factor is that ductus arteriosus need insert to uterine artery by ultraselection, the third is to control the dose of the drug. These factors need to be studied further. In addition, the feasibility of the operation again and the best time also need to be studied. Chen et al. [18] shows that the effect of injecting etoposide is better than that of MTX under the guidance of the peritoneoscope, which widen our thread when we select injecting drug.

In this study, the authors used clinical research regarding ultraselection uterine arterial cannulation to diagnose and treat tubal pregnancy, which can solve the problem of intraperitoneal hemorrhage, decrease toxic and side-collateral side-effects of injecting MTX, preserve the integrity of fallopian tubes and fertility function of the patient, and establish a new way to diagnose tubal pregnancy and synthetic expectant treatment.
References


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Folic acid and neural tube defects: are Jordanian pregnant women aware?

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Summary

Objectives: To assess Jordanian pregnant women’s awareness of folic acid and its contribution to neural tube defects (NTDs) prevention and to their folic acid intake. Materials and Methods: One thousand pregnant women were interviewed about their knowledge of folic acid for NTDs prevention and their folic acid intake using a questionnaire. Results: Of 1,000 women surveyed, 93.4% reported hearing of folic acid and 30.3% of NTDs. Only 16.2% knew that folic acid can reduce NTDs risk; 42.0% of those aware of folic acid believed it should be taken periconceptionally but only 16.9% did so. The most common information sources on folic acid were physicians (82.8%). Conclusions: Whereas a large percentage of pregnant Jordanian women were aware of folic acid, only a small proportion are aware that it prevents NTDs and should be taken periconceptionally. Also, there was a gap between awareness, knowledge, and intake of folic acid. Awareness and knowledge of NTDs was less prevalent among Jordanian women. Despite the efforts that have been undertaken, further effort is required to educate Jordanian women about folic acid contribution to NTDs prevention.

Key words: Folic acid; Jordan; NTDs prevention; Periconceptional; Trimester.

Introduction

Failure of accurate formation of the mammalian neural tube results in neural tube defects (NTDs) that may occur at the cranial and / or spinal levels and include a group of severe birth defects in humans [1]. There is no treatment for NTDs once the neural tube fails to close. Because neural tube closure is completed by day 28 of gestation in humans, prevention therapy must be initiated before and in the early days of pregnancy [2]. The benefits of prevention therapy were documented in trials of periconceptional multivitamin supplementation, utilizing women who had previously given birth to one or more infants with an NTD. Compared with 5.0% of the infants/fetuses of unsupplemented mothers, 0.6% of the infants/fetuses of the fully supplemented mothers had NTDs [3]. Prevention of the first occurrence of NTDs has also been seen using a multivitamin preparation containing folic acid prior to and during early pregnancy [4].

Clinical trials show that up to 70% of NTDs can be prevented by folic acid [5]. During the last few decades, there has been widespread awareness of the tangible role optimizing the levels of blood folic acid around the time of conception and during early weeks of pregnancy plays to minimize the possibility of having pregnancies affected by NTDs. This awareness has led to worldwide use of periconceptional folic acid supplementation following awareness campaigns in developed countries increasing folic acid use from 27% to 48% (UK), 5% to 36% (The Netherlands), 14% to 83% (Ireland), 19% to 43% (Western Australia), 32% to 40% (USA), and 17% to 28% (Canada) [6].

This shift in use frequency was accompanied by a shift in research orientations. Early research emphasized proving a positive effect for periconceptional intake on NTDs reduction or prevention. More recently focus has shifted towards understanding the best method to administer folic acid in the form of folic acid supplementation (400 µg /day) for all childbearing age women and four mg/day has been recommended in some countries for women who have already experienced an NTD-affected pregnancy [7].

The context of this study, Jordan, is a Middle Eastern country that has a population of approximately 5.5 million with a natural increase of 2.4%. It has a fertility rate of 3.5 (in urban areas) and 4.2 (in rural areas) with a birth rate of 29/1,000 population by 31.6 years as childbearing-women mean age [8]. Understanding Jordanian women’s awareness and intake of folic acid in addition to its contribution to NTDs prevention is of a paramount significance given three facts: (1) almost half of them (43.4%) do not use any family planning method, which indicates a high percentage of unplanned pregnancy [9]; (2) the NTDs rate, which reaches up to 3.8 / 1,000 [10], is higher than international incidence; and (3) pregnancy termination of fetal anomalies is prohibited by law in Jordan [11].

In addition to launching a national 1.5 ppm-level flour fortification plan in 2002 in Jordan [11], much effort is undertaken by Maternal Child Health (MCH) services to increase women’s health awareness. Nonetheless, health education services lack organization and comprehensiveness [12]. Particularly pertinent to the significance of folic acid's role in preventing NTDs is awareness of its importance in Jordan, as it is crucial for the health of future generations.
acid, there is little research, if any, to date that documents Jordanian women’s awareness level of this crucial issue. Undeniably, the worldwide public health campaigns to increase women’s awareness about folic acid are based on the premise that if women are well-informed about the gains of folic acid in preventing NTDs, the anticipated result is that they will follow the recommendations to take folic acid supplements [13]. Nonetheless, there is very little information available to determine whether the level of awareness Jordanian women have regarding folic acid significance goes in line with the current recommendations and whether they have a grasp of folic acid contribution to NTDs prevention.

There is an urgent need to better understand the awareness and behavior of Jordanian women regarding pregnancy and folic acid intake. This study is the first, to the best of the researchers’ knowledge, to assess Jordanian women’s knowledge and practice associated with folic acid at a wide scale using a representative sample from two major referral medical centers in one of the biggest cities in Jordan. Probably the closest study is that by Abu-Baker and Savage which was limited to awareness of folic acid by a small sample of 300 women [14, 15]. Similarly, it is a pioneer study in terms of linking pregnant Jordanian women’s awareness of folic acid to their awareness of NTDs. It is hoped that the results of this study will provide valuable information about Jordanian women’s level of knowledge regarding folic acid, its intake, and its contribution to NTDs prevention. This should help health promoters to develop focused awareness-spreading objectives.

Materials and Methods

The participants of the study were 1,000 women seeking routine prenatal care at two major referral medical centers in Amman (the capital of Jordan): (a) King Hussein Medical Center, which is the largest referral hospital in Jordan and one of the leading medical centers in the region [16] with more than 1,000 bed facilities to which all military and government insured patients are referred [17]; and (b) The University of Jordan Hospital, which is considered one of the oldest and largest hospitals in Jordan serving more than 0.5 million patients a year [18]. Inclusion in this study was limited to only pregnant Jordanian women in their third trimester.

A cross-sectional survey was carried out using face-to-face interviewer-administered questionnaire. The survey was carefully developed to ensure comprehensive coverage of both: (a) the characteristics of the participants and (b) the topic under investigation. The questionnaire was anonymous and on voluntary basis and developed by the researchers for the purpose of the study in light of a review of the extant literature. The questionnaire comprised three parts. Part 1 comprised of socio-demoraphic (age, occupation, residence, education, household income, etc.). Part 2 recorded clinical data (entry to prenatal care and total no. of pregnancies, etc.). Part 3 addressed participants’ awareness, knowledge about and use of folic acid, as well as awareness and knowledge of NTDs. For this study, awareness of folic acid was assessed by asking participants if they had ever heard of folic acid. When the response was “yes”, they were asked follow-up questions. These included identification of the appropriate time for taking folic acid and the time they commenced folic acid intake. They were also asked more questions about their knowledge of folic acid. This procedure extends to asking about awareness of NTDs; follow-up questions were asked only to those who reported “yes.” The face validity of the questionnaire was established through presenting it to a panel of three obstetrics and gynecology experts. Reliability was established through presenting the questionnaire to a group of women with the characteristics of the study population who were later excluded from the study, yielding a Cronbach alpha calculated coefficient of 0.85.

The data for this study was collected during the first three months of 2013. Following obtaining the IRB approval of the study instrument, women were approached in the two target medical centers during their waiting time to see the physician. Each participant was informed about the purpose of the study and a verbal consent was obtained from each prior to the interview. Collected data were fed into and analyzed for frequencies and percentages using the Statistical Package for Social Sciences (SPSS) 20.

Results

Table 1 shows the selected sociodemographic characteristics and clinical data of the interviewed women and the association with awareness, knowledge of the appropriate time for taking folic acid, and actual intake of supplemental folic acid. Among a total of 1,000 interviewed women, 93.4% reported that they had heard of folic acid.

Among the 93.4% who had heard of folic acid, the highest percentage was in the 25-29 year age group (98.3%) city residents (96.6%) with a diploma or higher (96.8%). Also, the percentage was higher (96.0%) among employed women (96.0%) with a household income of higher than 500 JD (96.2%) and two to three pregnancies (96.0%). Those who had entered the prenatal care after the first trimester were less aware of folic acid compared to those who entered before pregnancy (98.6%) or during the first trimester (93.8%).

Knowledge of the appropriate time for taking folic acid was associated with the age group of 25-34 years compared to younger or older women. High-income city citizens with higher education were also more knowledgeable, and so were those who had a higher number of pregnancies and entered in prenatal care before pregnancy. A higher periconceptional intake of folic acid was associated with the older age group (30 years and above). It was also associated with city residence, higher education, employment, higher income, more pregnancies, and earlier entry to prenatal care. Results of participants’ awareness of NTDs indicate that pregnant Jordanian women’s awareness of NTDs is higher among those educated (43.8%), employed (43.6%) woman with an age that ranges between 25 and 29 years (32.6%) living in a city (33.6%) with a household income higher than 500 JD (46.0%), with one pregnancy (34.2%). It was also higher among those who entered prenatal care periconceptionally (44.4%).
Table 2 presents participants’ responses on some selected questions pertinent to their awareness associated with what folic acid is. Asked to select one answer to the target question and in response to the question addressing their source of information about folic acid, no less than four-fifths (82.8%) reported it was from their doctors. Less contribution was reported to other sources (e.g., friend or relative, internet, or field of specialty), and the least was attributed to mass media (TV, radio, or magazines) (3.1% combined).

In response to what folic acid is, almost four-fifths (79.3%) believed it was a vitamin whereas one-fifth either reported a wrong answer or did not know. Additionally, no less than half (52.6%) of the women who were aware of folic acid believed it could be obtained from liver, green vegetables, and beans. Yet it was difficult to identify folic-fortified items (e.g., bread, water, salt, or rice) as more than four-fifths (83.4%) did not know, with the correct answer (bread) identified by only 7.6%. More than half (53.9%) of the participants were aware of folic acid contribution to congenital malformation but no less than one-third (36.4%) were not. Around 10% reported it contributes to protection from anemia and bone deterioration. As to the necessity of folic acid intake during pregnancy, the responses of the overwhelming majority (91.4%) was positive. The participants’ awareness of NTDs was relatively low; two-thirds (67.9%) reported they never heard of it. The perceived need for obtaining more information about folic acid was conceived by a very high percentage (94.2%) of the participants.

To gain a deep insight of pregnant Jordanian women’s knowledge of NTDs, further analysis addressed only those 303 who reported having heard of NTDs. Results (Table 3) indicate that less than half (44.9%) of them reported NTDs occurs during the first trimester compared to the second trimester (31.2%) or the third (3.3%). However, more than one-third (38.6%) did not know. In response to whether they had heard of any vitamin that can prevent NTDs in fetuses, the response of more than two-thirds (66.0%) was positive, which means that 200 out of the 303 who had heard of NTDs had also heard of a vitamin that can prevent it in fetuses.

When those 200 were asked to identify a vitamin (folic acid, Vitamin A, C, D, or E) that prevents NTDs in fetuses, four-fifths (81%) could identify folic acid as being the one. However, a much lower percentage (13.5%) selected other
vitamins, and a few (5.5%) reported they did not know. Of the 81% who linked folic acid intake to NTDs prevention, the majority (69.1%) began taking folic acid during the first trimester, others (29.0%) began periconceptionally, and few (1.9%) did not take it at all.

Discussion

In Jordan, where the NTDs incidence reaches up to 3.8 per thousand [10] with no possibility for pregnancy termination of fetal anomalies due to cultural values and law regulations [11], women’s knowledge of folic acid contribution to NTDs prevention is quintessential. While 93.4% of the present participants indicated that they had heard of folic acid, less than one-third had heard of NTDs, and 16.2% knew that it can reduce the risk of NTDs.

The awareness of folic acid among Jordanian pregnant women was comparable to that of women in some countries: 84.0% (USA) [19], 91.0% (Saudi Arabia) [20], 92.0% (Ireland) [21], and 95% (Canada) [22]. However, it is relatively higher than women’s awareness in some other countries (e.g., 22.0% (Turkey) [23], 35.8% (China) [13], 41.0% (UK), 46.6% (UAE), 54.0% (Qatar) [24], and 61.0% (Germany) [25]. Moreover, the intake of folic acid of Jordanian women who were aware of folic acid is higher than that in other countries (20.3% in Qatar) [24]. (33.0% in USA) [19], (57.0% in Scotland) [26], (67.9% in UAE) [27], and (68.0% in Spain) [13]. These findings are probably attributed to the fact that women in Jordan have a positive attitude toward folic acid, and when they are advised to take it, they do. Additionally, since the present sample was limited to those women who were seeking prenatal care, it is not surprising that their level of awareness was relatively high. This is especially true given the present results indicating that the majority of the women in this study began taking

Table 2. — Knowledge of interviewed pregnant women who ever heard of folic acid (n = 934).

<table>
<thead>
<tr>
<th>Question</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From which source did you get the information on folic acid?</td>
<td></td>
</tr>
<tr>
<td>TV and radio</td>
<td>14 (1.5)</td>
</tr>
<tr>
<td>Magazine</td>
<td>15 (1.6)</td>
</tr>
<tr>
<td>Doctor</td>
<td>773 (82.8)</td>
</tr>
<tr>
<td>Friend or relative</td>
<td>51 (5.5)</td>
</tr>
<tr>
<td>Internet</td>
<td>38 (4.1)</td>
</tr>
<tr>
<td>Field of specialty</td>
<td>43 (4.6)</td>
</tr>
<tr>
<td>Do you know what folic acid is?</td>
<td></td>
</tr>
<tr>
<td>Vitamin</td>
<td>741 (79.3)</td>
</tr>
<tr>
<td>Medicine</td>
<td>96 (10.3)</td>
</tr>
<tr>
<td>Mineral</td>
<td>34 (3.6)</td>
</tr>
<tr>
<td>Do not know</td>
<td>63 (6.7)</td>
</tr>
<tr>
<td>Which of the following foods are rich in folic acid?</td>
<td></td>
</tr>
<tr>
<td>Liver, green vegetables, and beans</td>
<td>491 (52.6)</td>
</tr>
<tr>
<td>Milk products</td>
<td>214 (22.9)</td>
</tr>
<tr>
<td>Do not know</td>
<td>229 (24.5)</td>
</tr>
<tr>
<td>Which of the following foods is fortified with folic acid in Jordan?</td>
<td></td>
</tr>
<tr>
<td>Bread</td>
<td>71 (7.6)</td>
</tr>
<tr>
<td>Water</td>
<td>27 (2.9)</td>
</tr>
<tr>
<td>Salt</td>
<td>19 (2.0)</td>
</tr>
<tr>
<td>Rice</td>
<td>38 (4.1)</td>
</tr>
<tr>
<td>Do not know</td>
<td>779 (83.4)</td>
</tr>
<tr>
<td>Have you heard about NTDs?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>300 (32.1%)</td>
</tr>
<tr>
<td>No</td>
<td>634 (67.9%)</td>
</tr>
<tr>
<td>Which of the following diseases is prevented by taking folic acid?</td>
<td></td>
</tr>
<tr>
<td>Protects from anemia</td>
<td>69 (7.4)</td>
</tr>
<tr>
<td>Prevents bone deterioration</td>
<td>22 (2.4)</td>
</tr>
<tr>
<td>Congenital malformation</td>
<td>503 (53.9)</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Do not know</td>
<td>340 (36.4)</td>
</tr>
<tr>
<td>Do you think it is necessary to take folic acid during pregnancy?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>854 (91.4)</td>
</tr>
<tr>
<td>No</td>
<td>80 (8.6)</td>
</tr>
<tr>
<td>Do you need more information about folic acid?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>880 (94.2)</td>
</tr>
<tr>
<td>No</td>
<td>54 (5.8)</td>
</tr>
</tbody>
</table>

Table 3. — Knowledge of interviewed pregnant women who ever heard of NTDs (n = 303).

<table>
<thead>
<tr>
<th>Question</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>During which trimester of pregnancy does NTDs occur?</td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td>136 (44.9)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>40 (13.2)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>10 (3.3)</td>
</tr>
<tr>
<td>Do not know</td>
<td>117 (38.6)</td>
</tr>
<tr>
<td>Have you heard about any vitamin that can prevent NTDs in fetuses?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>200 (66.0)</td>
</tr>
<tr>
<td>No</td>
<td>103 (34.0)</td>
</tr>
<tr>
<td>Which of the following vitamins can prevent NTDs in fetuses?</td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>162 (81.0)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>7 (3.5)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>15 (7.5)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Do not know</td>
<td>11 (5.5)</td>
</tr>
<tr>
<td>When did you start taking folic acid?</td>
<td></td>
</tr>
<tr>
<td>Before pregnancy</td>
<td>47 (29.0)</td>
</tr>
<tr>
<td>First trimester</td>
<td>112 (69.1)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Did not take folic acid</td>
<td>3 (1.9)</td>
</tr>
</tbody>
</table>

* Answered “Yes” when asked “Have you heard about any vitamin that can prevent NTDs in fetuses?” (n = 200)

b Specifically answered “folic acid” when asked “Which of the following vitamins can prevent NTDs in fetuses?” (n = 162)
Folic acid and neural tube defects: are Jordanian pregnant women aware?

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Admittedly, social marketing theory entails that people’s awareness and knowledge can be positively influ-

folic acid during, rather than before, pregnancy. Of no less importance than these possible explanations is the fact that this sample was drawn from Amman, the capital city of Jordan. Probably the level of awareness would be lower in rural areas, where 17.4% of Jordan population resides [28], with a high rate of physician attrition and replacement with inexperienced physicians [29].

Whereas the above findings relate to general awareness of folic acid and intake, specific details about awareness are equally important. Regarding the appropriate time of folic acid intake, the present results indicate that the percentage of Jordanian pregnant women who believed folic acid should be taken periconceptionally (42.0%) reflects a higher level of awareness compared to many other countries. This percentage, for instance, was 5.0% in Nepal [30], 7.0% in USA [19], 8.5% in Norway [31], 10.0% in Saudi Arabia [20], 22.0% in Germany [25], and 39.0% in South Australia [32].

Despite these promising findings, the present results suggest that only a low percentage (16.9%) of those who heard about folic acid had begun folic acid supplementation before conception. This percentage is not drastically different from the percentage in other countries. Examples include 8.1% (Mediterranean area) [33], 9.7% (Thailand) [34], 14.0% (Lebanon) [6], fewer than 15.0% (China) [13], 15.6% (Taiwan) [35], 17.0% (Oslo) [36], 18.0% (Ireland) [21], 20.12% (Iran) [37], and 21.0% (Scotland) [26].

According to this finding, it should be noted that although folic acid is recommended before conception [38], the present participants’ awareness of this crucial phase was relatively low, which has probably resulted in the low percentage of those who took folic acid periconceptionally. This finding might be attributed to several factors. First, married women typically visit obstetrics and gynecology clinics only in the case of conception failure or miscarriage incidents. Some do so only after they realize they are pregnant, which usually occurs after a month or so of pregnancy. Additionally, the major source of hearing about folic acid is physicians (82.8%), who are visited by a low percentage of women (7.2%) before pregnancy. One more possible explanation lies in the high percentage of unplanned pregnancies in Jordan [9], resulting in lack of specific information prior to conception.

The second major concern of the present paper, in addition to folic acid awareness, is assessment of Jordanian pregnant women’s knowledge of folic acid contribution to NTDs. First, general awareness of NTDs in the present sample (30.3%) is relatively lower than its counterpart in developed countries such as USA (45%) [39] and Canada (62.7%) [40], but higher than women’s awareness in some other countries, e.g., Nigeria (29.6%) [41]. Second, the percentage of those who could link folic acid to NTDs in the present study (16.2%) is comparable to the average percentage in other countries ranging between 13.0% and 17.0% [23, 42, 43]. The present findings suggest that those of this sample who visited physicians periconceptionally are the ones who were: (a) more aware of folic acid, (b) more aware of the appropriate time for taking it, (c) higher users of folic acid periconceptionally, and (d) more aware of NTDs. The authors’ experience suggests that Jordanian pregnant women are hesitant, if not reluctant, to take vitamin supplementation unless prescribed by a physician. Their delayed visits to doctors, during which they are informed about folic acid and its role in NTDs prevention, are probably the reason behind their delay in commencing folic acid intake. These results confirm previous findings reporting that in a study on 78 Jordanian mothers of neonates with NTDs in the north of Jordan, none took daily folic acid supplements prior to conception or during the first four weeks of pregnancy, nor did any attend antenatal clinic before less than six-week advance in pregnancy. This was attributed to the fact that it is not customary for women of reproductive age in Jordan to take folic acid supplements periconceptionally [44].

The present findings support research results indicating that despite folic acid significant contribution to eliminate NTDs, many periconceptional women are still not fully aware of its significance, and when they know some hardly follow supplementation recommendations to prevent NTDs [11]. This necessitates intervention. In USA, for example, only 52% of childbearing age women had heard of folic acid, 5% knew its contribution to preventing birth defects, and only 2% were aware it should be taken before pregnancy in 1995 [45]. Only two years after adopting an awareness-raising campaign, the percentage of those who heard of folic acid increased to 79% and those who distinguished its contribution to birth defects prevention reached 19% [45], and in 2005, awareness of folic acid reached 84%, with a lower percentage (19%) knowing it prevents birth defects [46]. The discrepancies between awareness, knowledge, and folic acid intake could be related to factors that stand behind behavioral change. Therefore, one way to spread awareness of folic acid significance is through including the problem of folic acid in the school curriculum, especially that of young women, in order to better reach the highest possible number of young women. In some countries, Germany for example, a teaching material entitled “Folic Acid and Pregnancy” has been prepared for this purpose. This can be extended to biology teachers [25]. Furthermore, continuous development and delivery of multi-level education strategies that emphasize timely-intake contribution of folic acid to NTDs prevention can also be beneficial. These can be accompanied by regular family planning consultations. Media should also participate in spreading awareness among women, particularly those unemployed, less-educated, village-resident women with an age younger than 30 years in low-income families.
enced by mass media, but their behavior change demands health professionals’ counseling intervention and interpersonal communication [47]. The present findings indicate heavy dependence on physicians in spreading folic acid awareness, with an increase in their undertaking this task [14]. This finding is not surprising given that Jordan, like many other Middle Eastern communities, still appreciates face-to-face communication. On the other hand, the role of mass media, as the present findings indicate is minimal, thus it has to be reconsidered.

Awareness-raising campaigns are also a necessity in Jordan, especially those targeting folic acid contributions to NTDs. Collaborative effort is required, therefore, from all stakeholder institutions and agencies to secure women’s awareness of taking folic acid prior to pregnancy. In addition to awareness raising, daily supplementation and fortification of food with folic acid should be considered as the best way to improve the balance of folic acid in women of childbearing age of this special population with consanguineous marriages comprise one-third to one-half of all marriages, with first cousin marriage rates in the range of 20–30% [48].

This study is not without limitations. It targeted only those women who were seeking prenatal care in two major referral hospitals in Amman, the capital city of Jordan. Therefore, the findings fall short behind representing those pregnant women who do not seek prenatal care or women in other areas of Jordan. In addition, since this study focused on only folic acid taken as supplementation, other folic acid delivery methods of folic acid are not included in this study. The time of data collection was during the work time, which might have influenced the percentage of the employed and unemployed pregnant women in this sample.

Acknowledgement
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References
Folic acid and neural tube defects: are Jordanian pregnant women aware?


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Clinical analysis of emergency peripartum hysterectomies in a tertiary center

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Summary

Objective: To investigate the incidence, indications, complications, and risk factors associated with increased mortality and morbidity of emergency peripartum hysterectomy (EPH). Materials and Methods: The authors retrospectively analyzed 48 cases of EPH performed within six-year interval at Ondokuz Mayis University Hospital. EPH was defined as the operation performed for life-threatening hemorrhage which could not be controlled with conservative treatment modalities within 24 hours of a delivery. Results: The incidence of EPH was 5.03 per 1,000 deliveries. The most common indication for EPH was abnormal placental adherence (n = 22, 45.8%), followed by uterine atony (n = 19, 39.6%). All the patients with placenta accreta had a history of repeat cesarian section (CS) and placenta previa. Total hysterectomy was performed in almost all of the patients (n = 47, 97.9%). All women required blood transfusions. Maternal morbidity was significant, with bladder injury (31.3%) and disseminated intravascular coagulation (18.7%) among the most common complications. There were one maternal (2.1%) and five neonatal deaths (10.4%). Conclusion: Since most of the EPH cases are associated with prior cesarean delivery, decision of the first CS should be made for true obstetrical indications. If conservative treatments fail to control massive obstetrical bleeding, blood products and an experienced obstetrician should be ready to perform EPH to decrease the maternal morbidity and mortality.

Key words: Emergency peripartum hysterectomy; Placenta accreta; Uterine atony.

Introduction

Emergency peripartum hysterectomy (EPH) is a life-saving procedure performed as a last resort for controlling massive obstetric hemorrhage when all conventional treatments have failed to achieve hemostasis. EPH is defined as a cesarean hysterectomy or hysterectomy performed within 24 hours of a vaginal or cesarean delivery. The incidence of EPH ranges between 0.2 and 5.4 per 1,000 deliveries [1, 2]. Due to the increasing rates of cesarean delivery and the developing pharmacologic agents to prevent atony, abnormal placentaion (placenta accreta and/or placenta previa) has replaced uterine atony as the most common indication for EPH [3].

The aim of this retrospective study was to investigate the incidence, indications, complications, and the risk factors associated with increased morbidity and mortality of EPH in a tertiary center.

Materials and Methods

Between June 2006 and June 2012, 48 cases underwent EPH at Ondokuz Mayis University Medical Faculty Hospital. Some of these patients were referred to the authors from other clinics due to intractable postpartum hemorrhage. This retrospective clinical study was approved by the local ethics committee of the hospital. Written informed consent was taken from all the patients before the operative procedures.

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The operation and pathology reports were used to determine the indication of hysterectomy. Duration of operation were obtained from anesthetic records. Febrile morbidity was defined as a temperature of 38°C measured at least 24 hours after hysterectomy and repeated at least once.

Statistical analyses were performed using Statistics Package for Social Sciences version 16.0. Each continuous variable was tested to check the normality distribution with the Kolmogorov-Smirnov test. All values were expressed mean ± SD unless stated otherwise.

### Results

During the six-year study period, a total of 9,535 women delivered at the present hospital; 1,803 (18.9%) of them delivered vaginally and 7,732 (81.1%) by CS. Forty-eight women were included who underwent EPH, giving a rate of 5.03 per 1,000 deliveries.

The mean age of the patients was 31.9 ± 5.2 years (range; 21-41). The median parity was 2.0 (range 0 - 4), and 91% of women (n = 44) were multiparous. The route of delivery was vaginal in seven patients (14.6%) and CS in 41 patients (85.4%). The mean gestational age was 36.4 ± 3.4 weeks (range 26 - 40) with a mean birth weight of 2,907 ± 821 grams (range 730 - 4,470). Total abdominal hysterectomy was performed in 47 cases (97.9%), whereas only one patient had subtotal hysterectomy (2.1%). The mean duration of surgery was 124.0 ± 44.4 minutes (range 40 - 240). The mean duration of postoperative hospitalization was 8.7 ± 5.6 days (range 4-29) (Table 1).

The indications for current CS were 32 repeat CS (78.1%), two abruptio placenta (4.9%), two fetal distress (4.9%), one breech presentation (2.4%), one placenta previa (2.4%), and three other indications (severe pre-eclampsia, eclampsia, and cephalopelvic disproportion) (7.3%). Twenty-seven patients (56.3%) were delivered at the present hospital, whereas 21 patients (43.7%) were referred to us from other clinics. The most common indication of EPH was abnormally adherent placenta (placenta accreta, increta or percreta) (n = 22, 45.8%), followed by uterine atony (n = 19, 39.6%). Other indications are shown in Table 2. Uterine atony was the most frequent indication (n = 2, 50%) in primiparous, whereas placenta accreta (abnormally adherent placenta) (n = 22, 50.0%) was more common, and only encountered in the multiparous. All the patients with placenta accreta (n = 22, 100%) had a history of repeat CS and placenta previa. Moreover, six of them (27.3%) had either a history of previous curettage or myomectomy operation. Twenty-five women (52.1%) had bilateral hypogastric artery ligation, and the procedure was more often performed in patients with placenta accreta (n = 15, 68.2%) than uterine atony (n = 5, 26.3%) which was statistically significant (p = 0.024).

Table 3 shows general characteristics and maternal and fetal outcomes of the present patients (patients delivered at the present center and evaluated at least once antenatally prior to the delivery) and the patients referred to our clinic from other hospitals. The most common indication of EPH was abnormally adherent placenta (74.1%) in the present patients, whereas it was uterine atony (76.2%) in patients referred from other hospitals (p < 0.001). The rate of bilateral hypogastric artery ligation, bladder injury, previous CS, and history of antepartum bleeding were significantly higher in our patients (p = 0.022, 0.025, 0.022, and 0.001, respectively), however, mean transfused blood products,
Clinical analysis of emergency peripartum hysterectomies in a tertiary center

gestational week, and fetal weight were significantly higher in referral patients ($p = 0.002, 0.001, \text{and } 0.001$, respectively). Preoperative hemoglobin values were significantly lower and the rate of disseminated intravascular coagulation (DIC) was significantly higher in referral patients ($p < 0.001; p = 0.022$, respectively). Demographic parameters, route of delivery, duration of surgery, and postoperative hospitalization were comparable between the groups ($p > 0.05$, Table 3).

Table 4 shows the complications associated with EPH. All women required blood transfusions. The most common operative complication was bladder injury in 15 patients (31.3%). Thirteen of these bladder injuries (86.7%) had occurred in patients with placenta accreta with a statistically significant difference than other indications ($p = 0.001$). Other complications were listed in Table 4. Four patients (6.3%) were re-operated due to ongoing intraperitoneal bleeding. All of these four patients were referred from other hospitals with the diagnosis of atony and three of them had also DIC. There was one maternal death (2%). She was delivered at outside clinic by CS for the indication of previous CS, and referred to the authors for postpartum atony and intractable bleeding. On admission, she had a hemorrhagic shock and secondary DIC. Although prompt resuscitation and EPH was performed at the present clinic, she died due to hemorrhagic shock and cardiac arrest just after the operation. Also, there were five neonatal deaths (10.4%): three of these cases were due to very preterm delivery and massive antenatal bleeding; one with placenta previa and accreta (26 week), two with abruptio placenta (26 and 29 weeks), fourth one was due to preterm delivery, severe pre-eclampsia, IUGR, and uterine atony (35 weeks) and the last one due to a complete uterine rupture at term.

**Discussion**

Despite advances in obstetrical care, postpartum hemorrhage continues to be the leading cause of maternal morbidity and mortality worldwide [4, 5]. EPH is usually performed to prevent maternal mortality when all conservative treatments failed to control massive obstetrical bleeding [6]. The overall incidence of EPH varies from 0.2

### Table 3. — Comparison of our patients with the patients referred from other hospitals

<table>
<thead>
<tr>
<th></th>
<th>Present patients ($n = 27, 56.3%$)</th>
<th>Referred patients ($n = 21, 43.7%$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.8 ± 4.6</td>
<td>30.7 ± 5.7</td>
<td>0.172</td>
</tr>
<tr>
<td>Gravidity</td>
<td>3.7 ± 1.4</td>
<td>3.4 ± 1.4</td>
<td>0.639</td>
</tr>
<tr>
<td>Parity</td>
<td>2.2 ± 1.0</td>
<td>2.1 ± 1.1</td>
<td>0.836</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>35.5 ± 3.6</td>
<td>38.2 ± 2.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Fetal weight (grams)</td>
<td>2,594.0 ± 753.1</td>
<td>3,429.3 ± 663.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Route of delivery (n, %)</td>
<td></td>
<td></td>
<td>0.110</td>
</tr>
<tr>
<td>Vaginal</td>
<td>2</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>CS</td>
<td>25</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Previous CS (n, %)</td>
<td>22/27 (81.5%)</td>
<td>10/21 (47.6%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Number of previous CSs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with a positive history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indication for EPH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormally adherent placenta (n, %)</td>
<td>20 (74.1%)</td>
<td>2 (9.5%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Uterine atony (n, %)</td>
<td>3 (11.1%)</td>
<td>16 (76.2%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Abruptio placenta (n, %)</td>
<td>2 (7.4%)</td>
<td>1 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>Placenta previa without accreta (n, %)</td>
<td>2 (7.4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Uterine rupture (n, %)</td>
<td>0</td>
<td>1 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>Cervical laceration (n, %)</td>
<td>0</td>
<td>1 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>10.6 ± 1.6</td>
<td>6.2 ± 1.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Postoperative</td>
<td>8.2 ± 1.6</td>
<td>8.4 ± 1.9</td>
<td>0.693</td>
</tr>
<tr>
<td>Transfused blood products (units)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>5.7 ± 3.8</td>
<td>9.8 ± 5.3</td>
<td>0.002</td>
</tr>
<tr>
<td>FFP</td>
<td>3.9 ± 6.6</td>
<td>10.6 ± 7.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TS</td>
<td>2.9 ± 8.2</td>
<td>4.9 ± 7.9</td>
<td>0.074</td>
</tr>
<tr>
<td>Operation time (minutes)</td>
<td>125.7 ± 48.5</td>
<td>122.2 ± 40.4</td>
<td>0.797</td>
</tr>
<tr>
<td>Hospitalization days (days)</td>
<td>9.2 ± 5.5</td>
<td>9.1 ± 3.7</td>
<td>0.500</td>
</tr>
<tr>
<td>Ligation of hypogastric artery</td>
<td>18/27 (66.7%)</td>
<td>7/21 (33.3%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Bladder injury</td>
<td>12/27 (44.4%)</td>
<td>3/21 (14.3%)</td>
<td>0.025</td>
</tr>
<tr>
<td>DIC</td>
<td>2/27 (7.4%)</td>
<td>7/21 (33.3%)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

CS: Cesarean section; EPH: Emergency peripartum hysterectomy; ES: Eritrocyte suspension; FFP: Fresh frozen plasma; TS: Trombocyte suspension; DIC: Disseminated intravascular coagulation.
In the study of Karayalcın et al. and/or placenta previa) in subsequent pregnancies [13, 14]. Previous cesarean delivery was associated with an increased risk of vaginal delivery [12]. In addition, other studies revealed that a previous cesarean delivery was associated with an increased rate of abnormal implantation. In subsequent pregnancies [13, 14, 15]. In the study of Karayalcın et al., 75.8% of patients with placenta previa and 75% of women with placenta accreta had previously had CSs [12]. In agreement with the recent literature, the authors found that placenta accreta (45.8%) was the most common indication for EPH. Furthermore, all the patients with abnormally adherent placenta (n = 22, 100%) had a history of repeat CS and placenta previa. Although indications of all cesarean deliveries were not shown, since the present hospital served as a last step in the obstetrical care network of the geographical area, the cesarean delivery rate was higher than the average of the country. Moreover, increasing nationwide CS rates led to an increase in the rate of abnormal implantation, which in turn gave rise to the present high rate of EPH compared with the literature [7, 8].

In the present study, 25 women (52%) had bilateral hypogastric artery ligation before proceeding to hysterectomy. In the study of Gayat et al., it was stated that abnormality of placental implantation increased sevenfold the risk of failure of either medical or surgical conservative management in patients with postpartum hemorrhage [16]. In agreement with this study, ligation of hypogastric artery was more often performed and failed in patients with abnormally adherent placenta (n = 15, p = 0.024). In the present series, total hysterectomy was preferred in most of the patients (97.9%) due to the high rate of placenta accreta. Similarly, Karayalcın et al. suggested to perform total hysterectomy in placental invasion anomalies instead of subtotal hysterectomy [13]. Due to the fact that subtotal hysterectomy is associated with a decreased risk of visceral injury, blood loss, short operating time, and hospital stay, most of the authors suggest subtotal hysterectomy for hemodynamically unstable patients [13, 14].

The main complications associated with EPH include blood transfusions, urinary tract injury, DIC, need for re-operation, febrile morbidity, and maternal death. In the present series, all women required blood transfusions, similar with the results of Kayabasoglu et al. [17]. However, the rate of the bladder injury (31.3%) which was the major operative complication of this study, was relatively high compared with the literature [18, 19]. In a systematic review by Rossi et al., the rate of urinary tract injuries was reported as 16% [20]. As previous uterine scarring and abnormally adherent placenta causes distortion of the pelvic anatomy and obliteration of the vesicouterine space, in the present study, most of the bladder injuries occurred in patients with abnormal placenta (13/15, 86.7%). The high rate of the patients with abnormally adherent placenta which in turn contributing to high total hysterectomy rate might have lead to the present high percentage of bladder injury. The maternal mortality rate in the present study was 2%, which was comparable with the results of Awan et al., and Kwee et al. [8, 9]. However Kwame-Aryee et al. and Fatima et al. reported a higher incidence of maternal mortality (12.9% and 8.7%, respectively) as the main indication for EPH was uterine rupture in their series [18, 21]. To decrease the operation time and the possible morbidities, the operation should be performed by an experienced obstetrician. However, in the study of Kwee et al., it was shown that a gynaecologist will on average encounter a peripartum hysterectomy once in 11 years. Therefore, the tertiary cen-

### Table 4. — Complications associated with emergency peripartum hysterectomy.

<table>
<thead>
<tr>
<th>Complication</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion</td>
<td>48 (100)</td>
</tr>
<tr>
<td>Bladder injury</td>
<td>15 (31.3)</td>
</tr>
<tr>
<td>DIC</td>
<td>9 (18.7)</td>
</tr>
<tr>
<td>Acute renal insufficiency</td>
<td>6 (12.5)</td>
</tr>
<tr>
<td>Febrile morbidity</td>
<td>5 (10.4)</td>
</tr>
<tr>
<td>ARDS</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Re-operation</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Hematoma of the subcutaneous tissue</td>
<td>3 (6.3)</td>
</tr>
<tr>
<td>Pulmonary infection</td>
<td>3 (6.3)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Maternal death</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>5 (10.4)</td>
</tr>
</tbody>
</table>

DIC: disseminated intravascular coagulation; ARDS: adult respiratory distress syndrome; Some of the patients have more than one complication.
ters or university hospitals should arrange post-graduate training courses regularly for at least one obstetrician attending from each hospital.

During the study period, 43.7% (n = 21) of the patients were referred to the present center from other clinics. Zeteroglu et al. reported a similar referral rate of 45.8% and all of the maternal deaths were seen in that group. They concluded that the mortality and morbidity of performing EPH was elevated in critical patients referred from other hospitals [7]. Similarly, the only maternal mortality in the present study was seen in a referred patient from another hospital. In addition, the comparison of the results of the present patients with the referred patients resulted in some positive and negative implications. Firstly, the main indication of EPH in the latter group was uterine atony (76.2%), and it can be speculated that the obstetricians had anticipated the complicated, hemorrhage-related conditions (placenta previa alone or combined with previous cesarean delivery, abnormally adherent placenta, etc.), and consulted them to the tertiary center before the onset of labor. However, the bad implication was that preoperative Hb values were significantly lower, and the mean transfused blood products were significantly higher in the referred group (p < 0.05). Delay in transport or proper management of postpartum hemorrhage had caused a higher blood loss and DIC rate, increased the need for transfusion, and the maternal morbidity and mortality.

A potential limitation of this study was the relatively small number of the EPH cases and its retrospective nature. However, the authors highlighted the significant complications of the procedure and its relationship with the previous cesarean delivery.

In conclusion, since most of the EPH cases are associated with prior cesarean delivery, the decision of the first CS should be made for valid clinical conditions. Identification of the risk factors, especially abnormal placenta, antenatally can aid in predicting which patients would need hysterectomy, so suitable patients could be referred to a tertiary center before the onset of labor. If an uncontrollable massive obstetrical hemorrhage occurs, obstetricians should apply standard conventional treatments immediately. If these attempts fail to achieve hemostasis, blood products and an experienced obstetrician should be ready to perform EPH to decrease the maternal morbidity and mortality.

References

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Premature ovarian failure: diagnosis and treatment

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Summary
Premature ovarian failure (POF), also known as primary ovarian insufficiency, is diagnosed in the event when primary or secondary amenorrhea and hypoestrogenism with high levels of gonadotropins occur in women before the age of 40. POF, caused by genetic, autoimmune and environmental factors, leads to a decrease in numbers of primordial follicles, accelerated atresia, and impaired follicular function. The diagnosis of POF is an indication for hormonal replacement therapy (HRT), which should be continued until the mean age of menopause in a given population. HRT reduces the intensity of vasomotor symptoms and has a beneficial effect on the central nervous, skeletal, cardiovascular, and urinary-reproductive systems.

Key words: Premature ovarian failure; Menopause; Hormonal replacement therapy.

Introduction
Premature ovarian failure (POF), also known as primary ovarian insufficiency, is diagnosed in the event when primary or secondary amenorrhea and hypoestrogenism with high levels of gonadotropins occur in women before the age of 40. It has been estimated that by the ages of 40, 30, and 20 approximately, one in 100, one in 1000, and one in 10,000 women, respectively, will have suffered from POF [1].

Etiology of POF
POF caused by genetic, autoimmune, and environmental factors, leads to a decrease in numbers of primordial follicles, accelerated atresia, and impaired follicular function. In case of spontaneous POF in women with normal karyotype, the loss of ovarian function is usually idiopathic (90% of the affected females). In the remaining cases, POF occurs due to genetic background: chromosomal aberrations (Turner’s syndrome, deletions and translocations of chromosome X, trisomy X, fragile X syndrome), single gene mutations (congenital glycosylation defects, galactosemia, blepharophimosis-postis-epicanthus inversus syndrome), type Ia pseudohypoparathyroidism, mutations of the follicle stimulating hormone (FSH) receptor, the luteinizing hormone (LH) receptor, and bone morphogenetic protein 15 [2, 3], or autoimmune background: antibodies against the pellicid zone, a-enolase, 21-hydroxylase, and 17a-hydroxylase, P450scc [1, 4-9].

Furthermore, POF may be the consequence of surgical interventions in the lesser pelvis, radiotherapy and chemotherapy, viral infections, and environmental factors. It is believed that any intervention in the lesser pelvis may increase the risk of POF as a result of changes in ovarian vascularization and inflammatory lesions. POF resulting from the excision of endometrial ovarian cysts, serous cysts or electrocoagulation of the ovarian surface in the course of polycystic ovary syndrome, has also been described. The risk is the highest in case of repeated surgeries of the ovaries. The incidence of POF after laparoscopic excision of bilateral endometrial cysts has been estimated to be 2.4% [10]. The POF ratio is higher also in women after hysterectomy as a result of changes in ovarian vascularization. As far as radiotherapy is concerned, POF is diagnosed in women who received a dose of 20 Gy [1], while the risk of POF after chemotherapy increases with patient age and dose, being the highest for alkylating agents and taxanes [1]. One month of chemotherapy is believed to shorten the reproductive period by 1.5 years [11]. POF may also be caused by some viral infections: parotitis, varicella, and cytomegalovirus [1], while the cause and effect connection with tuberculosis, malaria, and dysentery has not been extensively documented [3, 12]. Environmental factors that play a role in the etiology of POF include tobacco smoking (accelerates menopause by approximately 1.8 years), heavy metals, organic solvents, pesticides, and industrial chemicals.

POF diagnosis
As far as hormonal tests are concerned, apart from measuring FSH, LH, and 17b-estradiol concentration levels, it is recommended to assess the levels of prolactin (PRL), thyroid stimulating hormone (TSH), inhibin B and anti-Müllerian hormone (AMH). FSH result over 30 mIU/ml is diagnostic of primary ovarian failure. The diagnosis can be made if a two-fold increase in the FSH levels is noted twice every four weeks. A result over 15 mIU/ml is an indication for repeated testing and additional 17b-estradiol measurement. 17b-estradiol concentration over 50 pg/ml and/or LH levels higher than FSH signifies the presence of at least a few normal ovarian follicles. In women with POF, a decrease in the inhibin B levels usually precedes the increase of FSH concentration. Anti-Müllerian hormone is a more sensitive predictive marker of ovarian reserve than FSH. The presence of ovarian follicles on ultrasound is found in...
even 30% of women with spontaneous POF [14]. In 20% of them, the ovulation has been noted within four months of the observation and their chances for future pregnancy range from 5% to 10% [3].

Among all genetic tests, karyotype evaluation is recommended in all women with primary amenorrhea, as well as tests for fragile X mental retardation 1 (FMR1) gene in case of family history of POF, fragile X syndrome or mental retardation [13].

All women with spontaneous POF are recommended to undergo tests for anti-ovarian, anti-thyroid and anti-adrenal cortex antibodies. Hypothyroidism (25%), Addison’s disease (3%), and type I diabetes (2.5%) are common comorbid conditions for POF [22]. The presence of antibodies is especially prognostic in relation to adrenal cortex and correlates with future insufficiency of that organ. Thus, each woman found to have anti-adrenal antibodies is advised to undergo the corticotropin-releasing hormone (CRH, corticoliberin) test.

Laparoscopic ovarian biopsy is not recommended in POF women with normal karyotype. The result of the biopsy has no influence of the pregnancy prognosis (absence of follicles in the biopsy does not exclude their presence) and the procedure further diminishes the ovarian reserve. In some cases, ovarian biopsy allows to uncover the causes undermining POF. The presence of connective tissue but absence of ovarian follicles are found in case of gonadal dysgenesis, while autoimmune background will be demonstrated by the presence of perifollicular infiltrations consisting of lymphocytes.

**Prognosis**

The presence of ovarian follicles on ultrasound offers some chances for future pregnancy (5-10%) [3]. Other markers of good prognosis include unstable FSH levels and autoimmune and/or chemotherapy-related POF [24]. Primary amenorrhea remains to be the most significant adverse prognostic factor [15].

**Management**

**Hormone therapy**

The diagnosis of POF is an indication for hormone replacement therapy (HRT) that should be continued until a given population reaches menopausal age [16]. HRT reduces the intensity of vasomotor symptoms and has a beneficial effect on the central nervous, skeletal, cardiovascular, and urinary-reproductive systems. HRT has been confirmed to reduce the risk of changes connected with dementia, depressions, impaired cognitive functions and changes in the blood supply of certain parts of the brain. Such management improves bone density, lipid profile, the condition of the skin, and mucosa. 17b-estradiol (50 mg - injected or two mg - orally) and micronized progesterone (100 mg), dydrogesterone (ten mg) or norethisterone are used in sequential therapy. Androgen substitution is recommended in case of intensified symptoms of testosterone deficiency (absent libido) [13]. Unlike in women after menopause, no negative consequences or risks connected with HRT were found in POF cases.

**Contraceptives**

Hormonal contraceptives are not recommended due to changes that occur in the central nervous, cardiovascular, and urinary-reproductive systems. They are also believed to be ineffective because of high gonadotropin levels in POF women [23]. Barrier methods are preferable in POF.

**Infertility treatment**

Oocyte donation and in vitro fertilization is the method of choice in the infertility treatment of POF. That course of management is possible in all women with normal uterus and has a higher success rate than the standard in vitro procedure [25]. Other ways of management have been described in women who do not accept such management, among others didehydroepiandrosterone (DHEA) administration (50 mg/day for two to six months until conception) [17], pituitary gland suppression with estrogens, and ovulation induction with exogenous gonadotropins [18, 19]. Additionally, corticosteroid administration is recommended in autoimmune-related POF (dexamethasone one mg for three months) [20]. Recent years have brought reports on ovarian tissue or whole ovary transplants in women with POF [21].

**Conclusions**

POF may be caused by genetic and autoimmune factors, surgical interventions in the lesser pelvis, radiotherapy and chemotherapy, viral infections, and environmental stimuli. The diagnosis of POF is an indication for HRT that should be continued until a given population reaches menopausal age. Oocyte donation and in vitro fertilization is the method of choice in the infertility treatment of POF. Barrier methods are preferable contraceptives in POF.

**References**


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Manual versus computer-automated semen analysis

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Summary

Objective: To evaluate agreement of conventional sperm analysis with computer-aided semen analysis (CASA) regarding concentration, motility, and morphology using samples from infertile men. Materials and Methods: In this study a total of 195 male partners of couples who underwent evaluation of infertility were included. All semen samples were examined by conventional method and CASA in terms of morphology, motility, and concentration. Pearson correlation analysis and the Bland-Altman method were used to assess correlation and agreement between conventional semen analysis and CASA measurements. Results: When the two methods were compared in terms of concentration, motility, and morphology, there was a statistically significant correlation in all variables. The best correlation was obtained for sperm concentration. However, there was a poor correlation for sperm morphology between conventional method and CASA. Sperm concentration and morphology obtained by CASA were 14% and 87% lower, respectively; motility was 21% higher than the conventional method. Conclusion: Although CASA systems are objective and rapid, they should be evaluated in terms of cost-effectiveness, however they may be useful in over-loaded assisted reproductive technique (ART) clinics.

Key words: Sperm; CASA; Infertility; Assisted reproductive techniques.

Introduction

Abnormalities in sperm production or function, alone or in combination with other factors, account for 20-40% of all infertile couples [1]. After a detailed investigation with history and examination, semen analysis is generally demanded from male partners seeking fertility [2]. The findings of sperm analysis do not only clarify the etiology of infertility but also assist the management of treatment. Nevertheless, despite simplicity of semen analysis, careful analysis and a great deal of technical expertise is crucial for confidential results.

The method for conventional semen analysis is a somewhat subjective and time-consuming technique and inter-observer variability should not be disregarded [3]. Alternatively, computer-aided semen analysis (CASA) is developed to improve accuracy of sperm count and to establish a standardized method of test. However, the success of the technique is based on the advancement of technology, analytic conditions, and skills of the technicians. Additionally, the current data about their role for prediction of pregnancy either in natural conception or assisted reproduction cycles is still inconclusive [4].

The aim of current study is to evaluate agreement of conventional sperm analysis with CASA regarding concentration, motility, and morphology using samples from infertile men.

Materials and Methods

A total of 195 male partners of couples who underwent evaluation of infertility were included in this study. All semen samples were obtained in Hacettepe University, School of Medicine, Andrology Laboratory according to the local and World Health Organization (WHO) Laboratory Manual for the Examination of Human Semen and Cervical Mucus Interaction guidelines [5]. Specimens were obtained by masturbation after three to five days of sexual abstinence into sterile polypropylene container and allowed to liquefy for a minimum of 30 minutes at 37°C before semen analysis.

Conventional manual analysis was performed according to the methods described by the WHO guidelines [3]. Briefly, the concentration of spermatozoa was determined by using Makler counting chamber by single observer. Regarding the motility ratio, minimum of 200 spermatozoa were evaluated. Motility of each spermatozoon was classified as progressive motile, non-progressive motile, and immotile. Sperm morphology was assessed by Kruger’s strict criteria after observing 100 spermatozoa per slide [9].

Aliquots of the same semen samples were also examined with CASA for sperm concentration, motility, and morphology.

Statistical methods

SPSS 16.0 statistical analysis software was used to evaluate variables. Pearson correlation analysis was used to assess correlation between conventional semen analysis and CASA measurements. The Bland-Altman method was used to assess agreement between conventional semen analysis and CASA measurements. The p values less than 0.05 were considered as statistically significant.

Results

The mean male age was 31.6 ± 6.2 years and mean abstinence duration was four ± 1.4 days. The mean sperm volume was 3.7 ± 1.7 ml. The descriptive statistics of semen parameters assessed by conventional method and CASA are given in Table 1. When the two methods were compared in terms of concentration, motility and mor-
There was a statistically correlation between the two method's measurements in all variables (p < 0.001) (Table 1). The best correlation was obtained for sperm concentration. However, there was a poor correlation for sperm morphology between conventional method and CASA (Table 2).

Other than correlation analysis, when agreement was tested between two methods by Bland Altman, the mean biases (upper and lower limits of agreement) between conventional method and CASA according to sperm concentration, sperm motility, and sperm morphology are given in Table 2 and depicted in Figure 1. While sperm concentration and morphology obtained by CASA were 14% and 87% lower, respectively, motility was 21% higher than the conventional method.

Discussion

Conventional semen analysis with Makler chamber or hemocytometer is simple and routinely used test for the most of the andrology laboratories. Notwithstanding its simplicity, it shows intra- and interlaboratory variations. Therefore, there is a need to develop new methods to assess semen quality objectively.

Initially, CASA was developed to perform more accurate semen analysis. Its aim is to achieve standardized, objective, and reproducible test for sperm concentration, morphology, and motility. Use of CASA system in assisted reproductive techniques (ART) laboratories assumed to help in improvement of laboratory standardization and quality control studies. Besides these advantages, there are some disadvantages of CASA system such as the cost of the equipment and extreme need of validation.

There are some reports about CASA in andrology research and veterinary practise. Authors concluded CASA instruments were precise, efficient and reliable tool to evaluate fertility objectively and improve artificial insemination techniques [6, 7].

There are also a few studies in literature which compare these two semen analysis methods in human sperm. In one of them Cooper and Yeung compared two methods according to percentage of rapidly progressive (grade a) spermatozoa [8]. They reported that percentage of grade...
a spermatozoa from both methods were similar but did not agree well with each other. Vested et al. also compared the two methods in terms of sperm motility and concentration. They found the interclass correlation coefficients (ICC) for sperm concentration assessments was high (0.92), whereas there was no correlation for rapidly progressive and slowly progressive spermatozoa (ICC = 0) between the two methods. They also reported low correlation (ICC = 0.54) for non-progressive sperm between conventional and CASA assessments [9]. In another study, authors concluded that CASA system was able to provide sperm concentration and motility measurements which were at least as reliable as current manual methods [10].

In a recently published study, Lammers et al. reported that correlation coefficients of both automated systems with manual analysis were very high for sperm concentration, total sperm number, motile sperm concentration, and progressively motile sperm concentration. In same study concerning morphology, specificity (Sp), and negative predictive values (NPV) of CASA versus manual assessment were: SpCASA – 84%, NPVCASA – 96%, respectively. As a result; they concluded that automated sperm analysis systems can be considered as accurate tools for routine sperm analysis, providing high quality sperm analysis systems can be considered as accurate tools for routine assessment [11].

In the present study, sperm concentration, motility, and morphology results were markedly similar between conventional and manual methods (p < 0.001). However, ICC was found high (0.89) for sperm concentration and low for sperm motility and morphology, 0.62 and 0.44, respectively. The accuracy of sperm concentration appears to be diminished in the presence of severe oligospermia or excessive numbers of sperm [12, 13]. The high correlation found in the present study in terms of sperm concentration might be due to the small sample size or to the presence of phenominal severe oligopolyvespermia in the cohort.

Despite the similarity between two methods results, measurements of sperm concentration, motility, and morphology with CASA do not correlate very well with sperm motility and morphology measurements of manual methods.

In conclusion, although CASA systems are objective and rapid, they should be evaluated in terms of cost-effectiveness, however they may be useful in over-loaded ART clinics.

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Association study of vascular endothelial growth factor gene polymorphisms with ectopic pregnancy in Chinese women

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Summary

Objective: The purpose of this study is to evaluate potential associations between vascular endothelial growth factor (VEGF) gene polymorphisms and ectopic pregnancy (EP) in Chinese women. Materials and Methods: This was a case-control study wherein 192 women with a history of EP were compared to 210 post-menopausal controls with two pregnancies and no EP for the genotyping of VEGF polymorphisms. Genotyping of the VEGF gene polymorphisms at -460C/T, -1154G/A, -2578C/A and +936C/T were performed by polymerase chain reaction-restriction fragment length polymorphism. Results: No significant differences were found in genotype and allele distributions of the -460C/T, +936C/T polymorphisms between cases and controls. Compared with the -1154G/G genotype, the -1154A or -2578A alleles of VEGF gene could significantly decrease the risk of EP and might be potentially protective factors for EP development in Chinese women.

Key words: VEGF; Polymorphism; Ectopic pregnancy; Susceptibility.

Introduction

The incidence of ectopic pregnancy (EP) has increased and accounts for 2% of all pregnancies [1]. Ectopic pregnancy is a major cause of maternal morbidity and mortality, accounting for 9-13% of the first trimester pregnancy-related deaths [2]. It is an important cause of maternal deaths in early pregnancy because most fatal cases result from delayed diagnosis and inappropriate investigation [3]. In spite of high-resolution vaginal ultrasound and high sensitive quantitative beta human chorionic gonadotropin (beta-hCG) assays, at the first presentation, nearly 40-50% of EPs might be initially misdiagnosed [4]. For this reason, some serum markers have been investigated for early diagnosis of EP, such as, vascular endothelial growth factor (VEGF) [5].

VEGF is a well-known angiogenic factor, which may play a key role in the establishment of a viable pregnancy, participating in the processes of implantation and placentation [6], contributing to arterial remodeling and increase of permeability in endometrium, decidua, and trophoblast, leading to vascular development of the embryo [7,8]. The secretion and expression of VEGF is dependent on local conditions, such as hypoxia [9], and it has been observed that the cellular VEGF production is increased in hypoxic conditions [7,10]. The low basal expression of VEGF messenger RNA under normoxic conditions in the cytotrophoblast and the syncytiotrophoblast (in vitro) supports the lower levels of VEGF in patients with intrauterine pregnancy compared with that in patients with EP [11]. The production and secretion of VEGF seem to be elevated in EP because the implantation environment in the oviduct is very different from the well-vascularized endometrium [5, 7, 12]. Besides, several studies have demonstrated that VEGF seems to be an important serum marker for the diagnosis of EP [5, 12].

The VEGF gene is located in the chromosome region 6p21.3 and consists of eight exons and seven introns, which is a highly polymorphic gene, and a number of single nucleotide polymorphisms (SNPs) have been reported [13]. Numerous studies have shown that several polymorphisms were associated with the production of the VEGF protein, and suggested that the regulation of VEGF expression occurred primarily at a transcriptional level [13-17]. Four single nucleotide polymorphisms (-460C/T, -1154G/A, -2578C/A, +936C/T) of VEGF gene, which locate in the promoter and 5'- and 3'-untranslated regions, may alter VEGF expression [13-19]. Indeed, several studies have investigated the association between the above four single nucleotide polymorphisms and various diseases, including recurrent pregnancy loss, pre-eclampsia, and preterm delivery [20-22]. Considering the important roles of VEGF in pregnancy, the present authors are interested in studying the polymorphism of the VEGF gene, as this marker appears to be very important in the determinism of EP. Therefore they investigated whether -460C/T, -1154G/A, -2578C/A, and +936C/T VEGF polymorphisms alone or haplotypes with other VEGF polymorphisms were a risk factor for EP in a hospital-based case-control study.


Table 1. — PCR conditions for VEGF -2578C/A, -1154G/A, -460C/T, and +936C/T restriction fragment length polymorphisms.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer</th>
<th>Product length (bp)</th>
<th>Restriction enzyme</th>
<th>Fragment length</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF -2578C/A</td>
<td>5′-GGATGGGCGCTGACTGGTAAAGC-3′ (F)</td>
<td>324</td>
<td>BglII</td>
<td>324 (bpC)</td>
</tr>
<tr>
<td>rs999483</td>
<td>5′-AGCCCCCTTTTCTTCCACAC-3′ (R)</td>
<td></td>
<td></td>
<td>202 + 122 (bpA)</td>
</tr>
<tr>
<td>VEGF -1154G/A</td>
<td>5′-TCCTCTGCTCCCTCCTTCGCAGAATG-3′ (F)</td>
<td>206</td>
<td>MnlI</td>
<td>184 + 22 (bpA)</td>
</tr>
<tr>
<td>rs1570360</td>
<td>5′-GGCGGGAGAGGAGGACGGCAGCAG-3′ (R)</td>
<td></td>
<td></td>
<td>150 + 34 + 22 (bpG)</td>
</tr>
<tr>
<td>VEGF -460C/T</td>
<td>5′-TGTGCGTGTGGGTTGAGGCTGACG-3′ (F)</td>
<td>175</td>
<td>Bsh1236I</td>
<td>175 (bpT)</td>
</tr>
<tr>
<td>rs833061</td>
<td>5′-TACGGTGGGAGAACGGCGCTCTAG-3′ (R)</td>
<td></td>
<td></td>
<td>155 + 20 (bpC)</td>
</tr>
<tr>
<td>VEGF +936C/T</td>
<td>5′-AAAGAGGAAGAGGAGACCTCTGCCCAG-3′ (F)</td>
<td>198</td>
<td>Hsp92II</td>
<td>198 (bpC)</td>
</tr>
<tr>
<td>rs3025039</td>
<td>5′-TATGTGGGTGGGTGTGTCTACAG-3′ (R)</td>
<td></td>
<td></td>
<td>112 + 86 (bpA)</td>
</tr>
</tbody>
</table>

Materials and Methods

Study subjects

The case group comprised of 192 women with a history of EP (mean age 29.4 ± 4.3 years, mean amenorrhea 7.1 ± 2.6 weeks, previous EP 15.2%, and EP after in vitro fertilization 5.4%). The treatment of EP was: salpingectomy in 46.6%, salpingostomy in 12.4%, single dose of intramuscular methotrexate 50 mg/m² in 30.3%, and expectant management in 10.7% of the cases. These women were recruited from the Gynaecology Department of Zhjiang Hospital of Southern Medical University. The diagnosis of EP was performed by transvaginal ultrasound and confirmed during the surgery. The control group consisted of 210 healthy, post-menopausal women. The control group was matched with the case group for age, preeclampsia or preterm delivery. All case and control subjects were of Han nationality from South China. The Ethics Committee of Southern Medical University approved the study and informed consent was obtained from all recruited subjects.

DNA extraction

Venous blood (five ml) was drawn from each subject into Vacutainer tubes containing EDTA and stored at 48°C. Genomic DNA was extracted within one week after sampling using proteinase K digestion followed by a salting out procedure according to the previously described method [23].

VEGF -460C/T, -1154G/A, -2578C/A, +936C/T genotyping

Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCR was performed in a 20 µl volume containing 100 ng of DNA template, 1.6 µl of 10×PCR buffer for -460C/T (rs833061) and -1154G/A (rs1570360), 2.4 µl of 10×PCR buffer for -2578C/A (rs699947) and +936C/T (rs3025039), 1U of Taq DNA polymerase, 0.4 µl of ten mmol/l dNTPs, and 200 nM of each primer. The PCR cycling conditions were five minutes at 94°C followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 61°C for -460C/T and -1154G/A, 60.5°C for -2578 C/A, 63°C for +936C/T, and 45 seconds at 72°C, with a final step at 72°C for seven minutes to allow for the complete extension of all PCR fragments. PCR products (six to eight ml of reaction) were digested overnight at 37°C in a ten µl reaction with restriction enzyme as follows: 5U Bsh1236I for the -460C/T polymorphism, 5 U MnlI for -1154G/A, 5U Hsp92II for +936C/T and 10U BglII for -2578C/A. After digestion, the products were separated on a 4% agarose gel that was stained with ethidium bromide. The primers, length of PCR products, restriction enzymes and fragments length are summarized in Table 1.

For a negative control, distilled water was used instead of DNA in the reaction system for each panel of PCR. The PCR reactions of 15% of the samples were run in duplicate for quality control, with a reproducibility of 100%.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software package (version13.0, SPSS). Hardy-Weinberg equilibrium (HWE) analysis was performed to compare the observed and expected genotype frequencies using the Chi-square test. The data of age and amenorrhea weeks in the case group were presented as mean ±SD. Comparison of the VEGF -460C/T, -1154G/A, -2578C/A, and +936C/T genotype distributions in the study groups was performed by means of two-sided contingency tables using Chi-square test. The VEGF -460C/T, -1154G/A, -2578C/A, and +936C/T haplotype frequencies and linkage disequilibrium coefficient were estimated using the EH linkage software (version 1.2) and 2LD program, respectively. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. A probability level of 5% was considered significant.

Results

Association of VEGF -460C/T, -1154G/A, -2578C/A and +936C/T polymorphisms with the risk of EP

The distributions of the VEGF -460C/T, -1154G/A, -2578C/A, and +936C/T genotypes in the control groups did not significantly deviate from that expected for a Hardy-Weinberg equilibrium (all p values > 0.05). The genotype and polymorphic allele frequencies of the four polymorphisms among the cases and controls and their associations with EP risk are shown in Table 2. There were no significant differences in the genotype distributions and allele frequencies of VEGF -460C/T and +936C/T polymorphisms between the cases and controls (p = 0.564, 0.884 and 0.540, 0.294, respectively) (Table 2). Compared with the genotypes of -460C/T and +936C/C, the genotypes of -460C/C+C/T and +936C/T+C/T did not significantly influence the risk of EP (OR = 1.11, 95%CI = 0.74 - 1.66; OR = 1.22, 95%CI = 0.81 - 1.86, respectively) (Table 2).

The genotype frequencies of the VEGF -1154 A/A, G/A, and G/G in cases and controls were 2.1% / 6.2%, 26.6% / 33.8% and 71.4% / 60.0%, respectively; the A and G allele frequencies in the two groups were 15.4% / 23.1% and 84.6% / 76.9%, respectively. There was a significant difference in genotype and allele distributions of the VEGF -1154G/A between two groups (p = 0.021 and
The reason for studying a genetic predisposition for EP is that several patients who develop the disease do not present any risk factor. Besides, recent evidences have shown that VEGF values increase in EP when compared with that in normal intra-uterine pregnancy [5, 7, 10, 12]. Both aspects instigated the present authors to hypothesize that a polymorphism of the VEGF gene could be associated with EP. In this study, the four important functional polymorphisms of the VEGF gene -460C/T, -1154G/A, and -2578C/A were investigated, and which were selected for studying because they have a well-established laboratorial methodology and also because they present a direct correlation with the production of VEGF [13-19]. At present, the T/T, T/C, and C/C genotype of -460T/C SNP. d:

<table>
<thead>
<tr>
<th>Allele</th>
<th>C/T</th>
<th>T/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>60 (28.6)</td>
<td>55 (28.6)</td>
</tr>
<tr>
<td>T</td>
<td>250 (58.8)</td>
<td>180 (41.2)</td>
</tr>
</tbody>
</table>

**VEGF -1154G/A genotypes**

<table>
<thead>
<tr>
<th>Allele</th>
<th>G/A</th>
<th>A/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>100 (56.1)</td>
<td>150 (83.8)</td>
</tr>
<tr>
<td>A</td>
<td>70 (37.1)</td>
<td>20 (10.5)</td>
</tr>
</tbody>
</table>

**Discussion**

The four polymorphisms in the VEGF gene exhibit linkage disequilibrium with the other three polymorphisms. Therefore, haplotype analysis was only conducted between VEGF -460C/T, -1154G/A, and -2578C/A polymorphisms. The results of the EH linkage software analysis showed that the haplotype distributions derived from the three polymorphisms differed between the cases and controls (p = 0.000). The TGC(-460/-1154/-2578) was the most common haplotype in the controls (35.7%), followed by the CGC (17.6%), TGA (14.6%), TAC (9.6%), CGA (6.9%), CAC (6.4%), TAA (5.4%), and CAA (3.8%) (Table 3). Compared with the haplotype of TGC, the TAA, and CAA haplotypes could significantly decrease the risk of EP development (p = 0.020, OR = 0.41, 95%CI = 0.19 - 0.89; p = 0.014, OR = 0.29, 95%CI = 0.11-0.82, respectively) (Table 3). However, the others (CGC, TGC, CAC, TAA, and CAA) could not significantly modify the risk of developing EP (Table 3).
there has been no previous Chinese case-control study exploring this relationship.

In the present study, the results suggest that no significant association is found between the -460C/T and +936C/T polymorphisms and the risk of developing EP in Chinese women \( (p = 0.564 \) and 0.540, respectively). By contrast, a significant association is indicated between the VEGF-1154G/A, -2578C/A polymorphisms and the risk of developing EP in Chinese women \( (p = 0.201 \) and 0.034, respectively). Besides, the haplotype analysis suggests that the TAA(-460/-1154/-2578) and CAA haplotypes could significantly decrease the risk of developing EP compared with the haplotype of TGC in Chinese women \( (OR = 0.41, 95\%CI = 0.19 - 0.89; OR = 0.29, 95\%CI = 0.11 - 0.82; \) respectively).

Both the polymorphisms of -1154G/A and -2578C/A are located in the promoter region of the VEGF gene. The mechanism of VEGF-2578 is well known, which is in complete linkage with deletion/insertion of an 18-bp fragment at the -2549 region, and the construct containing the 18-bp deletion (linkage with the C allele) shows a 1.95-fold increase in transactivation [24]. VEGF -1154G/A is part of a predicted binding site for myeloid zinc finger-I (MZF1) in which the MZF1 binding site is substituted for the Pax2 or Sp1 binding site by -1154A. Considerable evidences have suggested that the two polymorphisms could affect the expression of VEGF, such as, Koukourakis et al. [25] have reported that the -1154G/G genotype in the VEGF gene was linked with protein overexpression of VEGF in non-small cell lung cancer; Shahbazi et al. [16] have also demonstrated that the -2578C/C and -1154G/G genotypes were correlated with higher levels of VEGF production by lipopolysaccharides (LPS) stimulated human peripheral blood mononuclear cells (PBMCs).

The -2578C/A and -1154G/A polymorphisms of VEGF gene have been investigated in a number of human diseases. However, the results are inconsistent. Some studies have shown that the VEGF -2578C/C and -1154G/G genotype were associated with increased risk of some diseases, such as acute rejection in renal transplant recipients [16], recurrent pregnancy loss [20], invasive breast cancer [26], and renal cell carcinomas [27]. On the contrary, other studies failed to show a significant association with diabetic retinopathy in type 2 diabetes [15] and severe preeclampsia [28]. However, the present results demonstrated that the -2578A/A+C/A and -1154A/A+G/A genotypes could significantly reduce the risk of developing EP in Chinese women, which were consistent with findings in other studies [29, 30]. This discrepancy might be explained by the following reasons: first, the frequencies of the VEGF-2578C and -1154G allele of the controls are different in the various populations. Such as the frequency of -2578C allele is 0.722 with the HapMap-HCB (Han Chinese in Beijing), and 0.592 with HapMap-CEU (Utah Residents with Northern and Western European ancestry); -1154G allele is 0.5-0.6 in Caucasians and 0.8 in Asians. The present data are 0.731(-2578C) and 0.769(-1154G) which are similar to that in China and Asians, respectively. It was validated that the present data were relatively reliable and indicated that ethnicity might play a critical factor in the manifestation of the effects of the polymorphic alleles. Second, previous evidences have shown that production and secretion of VEGF seemed to be elevated in EP [5, 12]. It has been manifested that VEGF-2578CC and -1154GG genotypes increase VEGF secretion compared with carriage of a rare allele (A; A) [16,19]. Therefore, these results further support the present authors’ conclusion that VEGF -2578A and -1154A allele may decrease the risk of EP development which is comparably reasonable. Perhaps, different molecular pathogeneses in different diseases might cause the discrepancy, which would contribute to further study.

The -460C/T is a common polymorphism in the 5′-untranslated region of the VEGF gene. The -460C/T and -405G/C polymorphisms of VEGF gene are in strong linkage disequilibrium and haplotypes containing -460C and -405G have a 71% higher basal promoter activity when compared with the wild-type sequence [18]. Whereas the effect of -460C/T polymorphism on the expression of VEGF remains unclear. Hsieh et al. [31] showed a significant association between -460C/T polymorphism and susceptibility to endometriosis, however, opposite results were reported by Zhao et al. [32]. In the present study, the -460 polymorphism did not modify the risk of EP development, in agreement with the report by Elito et al. in another study of Brazilian with EP [33]. These discrepancies may be due to sample size, participant selection criteria, difference in ethnicity or in the study design.

The +936C/T polymorphism locates on the 3′-untranslated region of the VEGF gene, and the +936T allele is related to low levels of VEGF plasma and the decreased risk of breast cancer [14]. The mechanism by which the VEGF +936T allele leads to lower VEGF plasma levels may be the loss of a potential binding site for activator protein 4 (AP-4) by the +936C->T transition in 3′-untranslated region and AP-4 is a helix-loop-helix transcription factor, enhancing expression of several viral and cellular genes by binding to specific enhancer sites; the loss of this potential binding site could be responsible for decreased VEGF expression by the T-allele [14]. Krippel et al. [17] have reported that the carriers of +936T allele was associated with decreased risk of breast cancer. However, the present study failed to show a significant relationship between the +936C/T polymorphism and the risk of developing EP. This result is consistent with previous published studies, which have shown no significant association of VEGF +936C/T polymorphism with EP in the Brazilian population [33]. Besides, the C and T alleles of +936 polymorphism were found at 84% and 16% in white population, respectively [14], frequencies similar to those found in the present study.

In addition, the present study has found that the three promoter region polymorphisms (VEGF -460C/T, -1154G/A,
and -2578C/A) were in linkage disequilibrium. The haplotype analysis suggested that the TAA (-460/-1154/-2578) and CAA haplotypes could significantly decrease the risk of developing EP compared with the haplotype of TGC. At present, no study has yet been published that investigated the effect of the haplotypes of VEGF -460/-1154/-2578 polymorphisms on VEGF production and their relationship to the development of EP. Therefore, further studies on the functional relevance of the VEGF polymorphisms and haplotypes in EP are required to confirm these results.

To the present authors’ knowledge, Elito et al. [33] were the first to demonstrate that there was no association between EP and -634C/G, -460T/C and +936C/T VEGF polymorphisms in the Brazilian population. Among them, the effects of -460T/C and +936C/T polymorphisms on EP in the Brazilian population are in agreement with the present results, but -1154G/A and -2578C/A polymorphisms show a significant association with EP of Chinese women in the present study. In comparison, the results of this study may be more reliable, the reasons are as follows: (I) in the present study, the authors included some important functional SNPs (-460C/T, -1154G/A, -2578C/A and +936C/T) in the genotype/haplotype analysis, and these tagging SNPs approach was used to cover the whole genomic region of the target gene (VEGF). Therefore, the present authors’ approach should allow to capture more comprehensive information on the VEGF gene block. (II) all participants were of Chinese ethnicity, because of its relatively homogenous ethnic origin, which stands in contrast with the more heterogeneous characteristics of the Brazilian women who were examined in the previous study. (III) the sample size was larger in the present study than in Elito et al. study: case group 192 vs 74, control group 210 vs 134.

From the present authors’ point of view, care should be taken to analyze these results, because of the limitation of the sample size, ethnic diversity, different compositions of case and control groups, the number of polymorphisms related to the gene in this disease, and whether confounding variables were considered. Nonetheless, further studies are required to replicate the present finding in different ethnic groups with a larger sample size.

In conclusion, the present authors conclude through their results that the -2578A or -1154A alleles of VEGF gene could significantly decrease the risk of EP development and might be potentially protective factors for EP development. Besides, the haplotypes of VEGF -460/-1154/-2578 polymorphisms may reduce the risk of EP development.

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Risk of pelvic floor dysfunctions in young athletes

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Summary

Purpose of investigation: Numerous epidemiological studies have shown a correlation between sport and the development of pelvic floor dysfunction. Therefore, the aim of the present study was to evaluate the prevalence of urinary incontinence in female young athletes. Materials and Methods: The epidemiological study was conducted on 105 female volleyball players, who were given a questionnaire, self-compiled, consisting of four main domains (personal data and medical history, urinary incontinence, urinary disorders, and judgment on the questionnaire). Results: In a total of 105 athletes, the present authors observed that 65.7% had reported at least one symptom of stress urinary incontinence (SUI) and/or urgency, during sport or in daily life situations. In particular, the 49.52% reported urge urinary incontinence, 20% urine loss for urgency, and 29.52% SUI. In addition, the present authors observed that nocturia was reported in 70.48% of cases, incomplete bladder emptying in 55.24%, urinary hesitancy in the 36.19%, and pelvic pain in 52.38%. In all cases, the symptoms were occasional and low. In relation to the coexistence of symptoms, the present authors observed that 22.85% of athletes had only symptoms of urge urinary incontinence, 6.66% mixed incontinence, and 6.66% symptoms of urge urinary incontinence associated to urine loss for SUI. Conclusion: The present authors observed a relationship between the sport and the pelvic floor dysfunction, in particular urinary incontinence.

Key words: Pelvic floor dysfunction; Urinary incontinence; Young athletes.

Introduction

Urinary incontinence is defined by the International Continence Society (ICS) as “a condition in which involuntary loss of urine is a social or hygienic problem and is objectively demonstrable” [1]. The most common type of urinary incontinence in women is stress urinary incontinence (SUI), defined as the involuntary loss of urine during coughing, sneezing, or physical exertion such as sporting activities, heavy lifting, or sudden change of positions. Genuine stress incontinence (GSI) is urodynamically proved involuntary loss of urine when the intravesical pressure exceeds that of the urethra with no simultaneous detrusor contraction. Urge incontinence is defined as involuntary loss of urine associated with a sudden, strong desire to void (urgency), and can occur alone or in combination with SUI (mixed incontinence) [1]. The most common type of urinary incontinence in women is SUI, followed by urge and mixed incontinence. Urinary incontinence is not a life-threatening or dangerous condition. However, it is socially embarrassing and may cause withdrawal from social situations and reduced quality of life [2, 3]. SUI implies that urine loss occurs during increases in abdominal pressure. If the condition is present, is therefore likely that urine loss will occur during physical activity [4]. The incidence of the urinary incontinence and consequently the susceptibility to urinary tract infections are increasing. Given the size of the problem, and considered that this dysfunction also affects young women and young athletes [5-7], the diagnostic [8, 9] and treatment of SUI has been the aspects of greatest interest for the urogynecological research [10-12]. Thus, sedentary women who are less exposed to physical exertion may not manifest stress incontinence, although the underlying condition may be present. SUI has shown to lead to withdrawal from participation in sport and fitness activities [13] and may be considered a barrier for life-long participation in health and fitness activities in women [14]. Strenuous physical activity has been suggested as one factor promoting pelvic floor dysfunction in women [15]. The pelvic floor muscles (PFM) comprise the pelvic diaphragm and the urogenital diaphragm. The muscles and fascias are important in giving structural support to all the internal organs and to close the pelvic openings. Pelvic floor dysfunction can cause urinary and fecal incontinence, pelvic organ prolapse, pain, and sexual disorders [16]. There are two opposing hypotheses about how strenuous exercise or hard work might affect the pelvic floor: physical activity may strengthen the PFM and physical activity may overload, stretch, and weaken the pelvic floor [17]. However, little is known about the direct impact of physical activity on pelvic floor anatomy and function. Ree et al. [18] found that there was a short-term fatigue of the pelvic floor muscles in young nulliparous women with symptoms of SUI after 90 minutes of strenuous physical exercises, but concluded that further research was needed to understand the long-term effects. O’Dell et al. [19] measured vaginal pressures during different exercises and found...
that the exercises studied generally produced lower pressure than cough, but individuals varied in pressure exerted. In addition, although the prevalence of urinary incontinence is high among female athletes, to date, there is scant knowledge about the long-term effect of strenuous physical activity on the prevalence of urinary incontinence [20]. In addition, there are very few studies on risk factors of urinary incontinence in elite athletes. Hence, the aim of the present study was to investigate whether former female elite athletes are more likely to experience urinary incontinence later in life than non-athletes and to assess possible risk factors for urinary incontinence in elite athletes. The aim of this study was to elaborate on the problem of urinary incontinence among female volleyball players while participating in their sport and during daily life activities.

Materials and Methods

A questionnaire about urinary symptoms was distributed to female volleyball players in nine different clubs. The present authors enrolled athletes ≥ 16 years of age and who were undergoing three weekly training sessions of two hours each. A total of 105 women were included in the study; all the women answered the questionnaire. The women were first asked if they experienced urine loss while participating in their sport or in daily life. If their answers to both these questions were negative, they did not complete the rest of the questionnaire. All women who had experienced urine leakage completed the questionnaire. They were asked about medications, deliveries, incontinence during training, competition and daily life activities, incontinence treatment, pad use, and voiding habits. The present authors evaluated the frequency of individual symptoms, the association of multiple symptoms, years of sport, and hours of weekly training.

The study was approved by the local scientific ethical committee and informed consent was obtained from all the women. Data analysis was performed using use of SPSS version 15. Data are presented as means with standard deviation (SD), frequencies, and percentages.

Results

The average age of the 105 athletes answering the questionnaire was 21.96 years (± 5.6) and they were nulliparous. The average BMI of the 105 athletes answering the questionnaire was 22.50 (± 4.2). Sixty-nine athletes (65.71%) were undergoing to ≥ eight but ≤ ten hours of weekly training. The remaining women are undergoing to ten hours of weekly training. Fifty-seven women (54.59%) practiced volleyball for more than ten years. All the women had a negative medical history for chronic diseases and surgeries and were not taking medication.

A total of 69 (65.7%) had reported at least one incontinence symptom while participating in their sport or in daily life situations, or urge incontinence or stress urinary incontinence or mixed type of incontinence. A total of 42 (40%) used pads during the competitions.

In particular, in relation to each specific symptom, 49.52% (52) reported urge urinary incontinence, 20% (21) reported urine loss for urgency, 29.52% (31) reported urine loss for SUI. Furthermore the present authors observed that the nocturia was reported by 74 athletes (70.48%), the feeling of incomplete bladder emptying was reported by 58 athletes (55.24%), and urinary hesitation was reported by 38 athletes (36.19%), and pelvic pain was reported by 55 athletes (52.38%). The athletes reported that these symptoms were occasional.

In relation to the coexistence of symptoms, the present authors observed that 24 (22.85%) athletes reported only urge urinary incontinence symptoms, seven (6.66%) mixed incontinence, and seven (6.66%) reported urge urinary incontinence symptoms associated to urine loss for stress urinary incontinence.

The present authors observed that any athlete had pure urge urinary incontinence and any coexistence of symptoms of urge and SUI. The SUI was caused by laughter and physical activity, respectively, in 35.45% in the first case and in the 32.26% in the second case.

The frequency of loss urine was once a month in the 23 women (74.19%) who reported SUI and in 17 women (80.95%) who reported urge urinary incontinence. This urine loss was defined low in quantity.

The present authors evaluated the frequency of urinary incontinence in relation to the years of playing volleyball. They observed in 57 athletes who played volleyball for more than ten years: 25 athletes (43.85%) had no urge and/or SUI, 13 athletes (22.8%) reported urge urinary incontinence, Nine athletes (15.78%) reported SUI, five athletes (8.77%) reported urge and SUI, and five (8.77%) reported urgency and SUI. Instead, the present authors observed in the 35 athletes who played volleyball for more than five years: seven athletes (20%) had no urge and/or SUI, nine athletes (25.71%) reported urge urinary incontinence, eight athletes (22.85%) reported SUI, eight athletes (22.85%) reported urge and SUI, and three (8.57%) reported urgency and SUI.

In 13 athletes who played volleyball for less than five years: four athletes (30.76%) had no urge and/or SUI, three athletes (23.07%) reported urge urinary incontinence, three athletes (23.07%) reported SUI, two athletes (15.38%) reported urge and SUI, and one (7.69%) reported urgency and SUI.

Finally the present authors evaluated the frequency of urinary incontinence in relation to the hours of weekly training. They observed in the 69 athletes who played volleyball for less than ten hours/week: 27 athletes (39.13%) had no urge and/or SUI, 13 athletes (18.84%) reported urge urinary incontinence, 12 athletes (17.39%) reported SUI, ten athletes (14.49%) reported urge and SUI, and seven (10.60%) reported urgency and SUI.

Instead, the present authors observed in the 36 athletes who played volleyball for more than ten hours/week: nine athletes (25%) had no urge and/or SUI, 11 athletes (30.55%) reported urge urinary incontinence, five athletes...
(13.88%) reported SUI, four athletes (11.11%) reported urge and SUI, and seven (19.44%) reported urgency and SUI. The results are summarized in Figures 1 and 2.

Discussion

The present study shows results in agreement with the studies in the literature that have investigated the prevalence of urinary incontinence in female athletes. The present results showed leakage of urine essentially due to stress occurring less than once a month and in small quantities. The problem of urinary incontinence in female athletes is still not widespread and the athletes live the problem with superficiality, this for the infrequency with which it occurs and for the low level of losses. This leads to a delay in diagnosis and a lack of knowledge of the potential for treatment and prevention. The approach considered as a first choice for prevention is pelvic floor muscle training (PFMT); this treatment has scientific evidence level 1 and Grade A and devoid of adverse effects. It would be appropriate therefore to subject athletes to PFMT to prevent the onset of urinary incontinence; it is absolutely necessary that the training is specific for the muscles of the pelvic floor. The results of the present study demonstrated a high prevalence of both SUI and urge incontinence in young female athletes. Bo et al. [21, 22] reported 26% prevalence in young physical education students, and Nygaard et al. [20] reported 28% in college athletes. According to the ICS definition, urinary leakage should be objectively demonstrable to fulfil the criteria of incontinence. This requires urodynamic assessment, and is not possible in the setting of an epidemiological study. Whether the subjects responding to the questionnaire in the present study had stress or urge urinary incontinence can therefore not be confirmed, and the results must be interpreted with caution. Urinary incontinence is not a life-threatening condition. However, it has been shown to affect quality of life, participation in social activities, and self-esteem. In elite athletes, it may affect concentration and performance, especially in sports with minimal and tight clothing such as figure skating and gymnastics. The high prevalence reported in aesthetic sports may reflect that the condition is visible and easier to recognize in those sports.
Factors contributing to urinary incontinence in young nulliparous women are not fully understood. Weak connective tissue combined with high-intensity and high-impact activity may unmask the condition [7]. Nygaard et al. [20] demonstrated that in 17% of women, the condition was only recognized during physical activity. Davis and Goodman [23] studied 512 of 2,651 female soldiers who entered the airborne infantry and found that nine developed urinary incontinence during the training period. Urodynamic investigation demonstrated detrusor instability in three, and GSI in six of the women. Four of the women reported feeling a tearing pain in their “lower quadrant” on impact during parachute jump, and one subject related a similar event during heavy lifting and doing sit-ups. However, 9 of 512 is a very low incidence. In addition, parachute jumping is an extreme high-impact activity.

The present study demonstrated that more than 60% of female volleyball athletes have experienced urine loss. Only the incontinent women completed the questionnaire, for which reason the authors cannot make comparisons between incontinent and continent athletes. In particular 65.71% of the athletes who had experienced urine leakage considered it to be a social or hygienic problem, and 40% occasionally wore pads. Thus physical exertion seems to be a significant provocative factor. Nygaard [20] reported that 20% of young women exercisers stopped because of urine leakage. Consequently, the frequency of regular urinary incontinence in a ‘normal population’ seems to be significantly higher than the findings in the present population of elite athletes and dancers. The activity most likely to provoke leakage was jumping. This explains why gymnastics, which involves many high-impact jumps, has the highest degree of leakage of the different sports. The study demonstrates that significantly more athletes experienced urine loss during training rather than competition: 95.2% versus 51.2%, respectively. This is an interesting finding that may be explained by the higher catecholamine levels during competition versus training [20]. As the urethra contains $\alpha$-receptors, the higher catecholamine level during competition may tend to keep it closed. Other factors, such as the ritual bladder and bowel emptying which is common before competition, or changes in diuresis, may also play a role.

The present results, in accordance with literature, provide more evidence as to the surprisingly high prevalence of urinary incontinence in young, physically fit athletes. Although relatively few women experienced frequent symptoms, we traditionally would not expect any incontinence at all in this group. It seems that the relatively high pressure generated by certain activities, especially jumping and ball games, is sufficient to overcome the continence mechanism in these women. Now that we know the prevalence of incontinence is higher in athletes than previously suspected, the next step will be investigating the pathophysiology. Do repeated impacts somehow damage pelvic supporting structures? Despite their overall level of physical fitness, is their pelvic muscle function abnormal for some reason? Just as isolated enzyme deficiencies have led to a greater understanding of cellular biochemistry, out of proportion to the actual incidence of those clinical conditions, unraveling the pathophysiology of incontinence in unusual study populations may help advance our understanding of continence and incontinence. The rationale would be that any physical activity that increases abdominal pressure will lead to a simultaneous or pre-contraction of the PFM, and the muscles will be trained. Based on this assumption, general physical activity would prevent and treat SUI. However, women leak during physical activity and they report worse leakage during high-impact activities. No sports involve a voluntary contraction of the PFM. Many women do not demonstrate an effective simultaneous or pre-contraction of the PFM during increased abdominal pressure. In nulliparas this may be due to genetically weak connective tissue, location of the PFM at a lower, caudal level inside the pelvis, lower total number of muscle fibres (especially fast twitch fibres) or untrained muscles in those leaking. To date, there is little knowledge about PFM function in elite athletes. Bo et al. [24] measured PFM function in sport and physical education students with and without urinary incontinence and did not find any difference in PFM strength. The increase in PFM pressure during a voluntary contraction was 16.2 cm H$_2$O in the group with SUI and 14.3 cm H$_2$O in the continent ones. However, this study was limited by its small sample size and no strong conclusion can be drawn. Statistically significant differences in PFM function and strength between continent and incontinent women have been shown in the adult population [25]. Bo et al. assessed PFM strength in four elite female power lifters and compared them to 20 physical therapy students. Mean muscle strength during voluntary contraction in power lifters was 22.6 cm H$_2$O and in the physical therapy students it was 19.3 cm H$_2$O which was not significant. Only one of the elite athletes in the above-mentioned ongoing study had exercised the PFM systematically. She reported to have trained her PFM regularly in order to increase low back stability and abdominal pressure during lifting. Her mean PFM strength was 36.2 cm H$_2$O. She was totally continent even when competing in World championships, but so too were those who had not trained the PFM [24].

Heavy lifting and strenuous work have been listed as risk factors for the development of pelvic organ prolapse and SUI. Nichols and Milley [26] suggested that the cardinal and uterosacral ligaments, PFM, and the connective tissue of the perineum might be damaged chronically because of repeated increases in abdominal pressure due to hard manual work and chronic cough. To date, there are still little data to support the hypothesis. In a study of Danish nursing assistants, it was found that they were 1.6 times more likely
to undergo surgery for genital prolapse and incontinence than women in the general population [27]. However, the study did not control for parity and, therefore, it is difficult to conclude whether heavy lifting is an aetiopathological factor. Twenty-six percent of women in the US Air Force female crew, capable of sustaining up to 9G, reported urinary incontinence [28]. However, more women had incontinence off duty than while flying and it was concluded that flying high-performance military aircraft did not affect the rate of incontinence. Davis and Goodman [23] found that nine out of 420 nulliparous female soldiers entering the airborne infantry training programme developed severe incontinence. Hence, most women were not negatively affected by this high-impact activity. Hay [29] reported the maximum vertical ground reaction forces during different sport activities to be: three to four times bodyweight for running, five to 12 times for jumping, nine times for landing from front somersault, 14 times for landing after double-back somersault, 16 times during landing in long jumps, and nine times bodyweight in the lead foot in javelin throwing. Thus, one would anticipate that the pelvic floor of athletes needs to be much stronger than in the normal population to counteract these forces. To date, it has been concluded that there is no evidence that strenuous exercise causes SUI or pelvic organ prolapse. Although the prevalence is high, most athletes do not leak during strenuous activities and high increases in abdominal pressures. However, from a theoretical understanding of functional anatomy and biomechanics, it is likely that heavy lifting and strenuous activity may promote these conditions in women already at risk, e.g. those with benign hypermobility joint syndrome. Physical activity may unmask and exaggerate the condition. From the data available today, it is not possible to conclude whether high-impact activity itself can cause connective tissue or PFM damage. In a retrospective study of former female Olympians competing in either low- (swimming) or high-impact (gymnastics) activities, no difference in prevalence in urinary incontinence was seen after 20 years of cessation of the sport carrier [23]. It was concluded that participation in regular, strenuous, high-impact activities when younger did not predispose for significant urinary incontinence later in life. In a consensus statement from the first WHO Consultation on Incontinence [29], it was concluded that strenuous exercise is likely to unmask the condition in otherwise asymptomatic women. However, there is no evidence that strenuous exercise causes the condition of incontinence. Evidently, more basic research is needed to understand the function and role of the pelvic floor during strenuous physical activity. A higher prevalence of both SUI and urge symptoms was demonstrated in eating disordered athletes than healthy athletes. Hextall et al. [30] compared 30 women with anorexia nervosa (mean age, 26.5 ± 7.3) with 25 healthy age-matched controls, and found that 93% of the women with anorexia nervosa had one or more urinary symptom compared with 36% of the control group ($p = 0.01$). The prevalence of both SUI and urge incontinence was 41%.

The female lower urinary tract is a target site for sex steroids and sensitive to fluctuations in the level of circulating estrogen and progesterone. However, the association between low estrogen levels and prevalence and degree of SUI and urge incontinence is not clear. Nineteen percent of the athletes compared with 37 (9%) of non-athletes reported that they were using oral contraceptive pills to regulate their cycle. This may have influenced the results. More research is needed on hormonal status in this specific group of female athletes. Since there is a lack of longitudinal studies in this area, it is difficult to draw any conclusion on incidence, remission, and natural history. Remission of one-third of females with urinary incontinence has been reported in older women. However, whether this is because of, for example, medical care, reduction of participation in physical activity, or unreliable measurements is difficult to say. Urinary incontinence can be treated with PFM exercise with and without biofeedback, electrical stimulation, or surgery. Randomized controlled trials have demonstrated that PFM exercise is significantly more effective than no treatment and more effective than electrical stimulation. Miller et al. [31] demonstrated that simply teaching women to voluntarily contract before and during coughing significantly reduced the leakage after only one week of practice. Bø et al. [21] demonstrated that after specific strength training of the PFMs, 17 of 23 women had improved during jumping and running, and 15 during lifting. In addition, significant improvement was obtained while dancing, hiking, during general exercise classes, and in overall score on ability to participate in different activities. Measured by pad test with standardized bladder volume comprising running, jumping jacks, and sit-ups, there was a significant reduction in urine loss from a mean of 27 g (95% Cl, 8.8–45.1) to 7.1 g (95% Cl, 0.8–13.4) ($p = 0.01$). So far there are no randomized controlled trials assessing the effect of PFMT in female elite athletes. Elite athletes are motivated and used to regular training. Since most of these athletes are nulliparous, there are no ruptures of ligaments, fascias, and PFM fibers, or peripheral nerve damage caused by pregnancy and childbirth. One may therefore expect the strength training to be equally or even more effective than in parous women. PFM exercise is non-invasive, has no known side effects, and can be very cost-effective (especially when combining individual assessment and group training), and is suggested as the first treatment option. There is a need for randomized controlled trials to evaluate the effect of strength training of the PFMs in this group of women. Devices that involve external urinary collection, intravaginal support of bladder neck, and blockage of urinary leakage by occlusion at the external meatus or intraurethral occlusion are available, and can be recommended to be used during physical exertion.
Conclusion

The results of the present study indicate that almost 60% of volleyball players surveyed reported a problem of urinary incontinence. Therefore, urinary incontinence is a common symptom among athletes but still too underestimated. We need to give a greater awareness of the problem that is the athletes and athletic trainers and it is necessary to introduce the specific PFMT to prevent the onset of urinary incontinence among young athletes.

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Role of levonorgestrel-releasing intrauterine system in dysmenorrhea due to adenomyosis and the influence on ovarian function

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Summary

Background: The objective of this study was to evaluate the efficacy and side-effects of the levonorgestrel-releasing intrauterine system (LNG-IUS) in the treatment of moderate or severe dysmenorrhea associated with adenomyosis and the influence on ovarian function. Study Design: The LNG-IUS was inserted into 60 women who had moderate or severe dysmenorrhea associated with adenomyosis diagnosed by transvaginal sonography. A visual analogue scale (VAS) of dysmenorrhea, uterine volume and serum-levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and prolactin (PRL) were used to assess the efficacy of the treatment at baseline and at six and 12 months after the LNG-IUS. Serum-levels of FSH, LH, E2, and PRL were tested in pre-and post-insertion at six and at 12 months, respectively. Side-effects were recorded at every follow-up visit. Results: After six and 12 months of LNG-IUS insertion, dysmenorrhea was obviously alleviated, and the dysmenorrhea scores decreased to 2.6 from 0.6 (p < 0.05). The volume of uterus reduced six months after insertion and later, but without significant change (p > 0.05). After treatment of serum, in terms of FSH, LH, and E2 levels, compared with pre-insertion, there was no statistically significant difference (p > 0.01). However, the level of PRL markedly declined at six and 12 months after LNG-IUS. Conclusion: The LNG-IUS appears to be an effective method in alleviating dysmenorrhea associated with adenomyosis with little effect on ovarian function. It may be helpful to decrease the level of PRL in these patients.

Key words: Adenomyosis; Dysmenorrhea; Levonorgestrel-releasing intrauterine system.

Introduction

Adenomyosis is a common disorder that affects more than five percent of women in their reproductive age [1] and is one of the most common causes of dysmenorrhea. Women suffering from moderate or severe dysmenorrhea are often under mental stress before their expected menses. Until recently, the only definitive means of diagnosing and curing adenomyosis was hysterectomy [2, 3]. The recent development of non-surgical diagnostic techniques, such as transvaginal sonography (TVS) and magnetic resonance imaging (MRI), makes it possible for gynecologists to investigate conservative treatment to manage dysmenorrhea associated with adenomyosis [4, 5]. The levonorgestrel-releasing intrauterine system (LNG-IUS) was initially devised for contraception. It releases 20 mcg/day of LNG into the uterine cavity for a five-year period [6]. Besides the high contraceptive efficacy of the LNG-IUS, it has been demonstrated that the device also benefits women in other aspects, such as symptoms control with endometriosis and adenomyosis [7-11]. Compared with the studies on the efficacy in the treatment of dysmenorrhea, there has been limited literature regarding the influence of ovarian function after LNG-IUS insertion. The objective of this study was to evaluate the efficacy, side-effects, the influence on ovarian function, and acceptability of the LNG-IUS in the treatment of moderate or severe dysmenorrhea associated with adenomyosis.

Materials and Methods

Sixty women aged between 30 and 45 years with complaints of moderate or severe dysmenorrhea were recruited to participate in this study. This study was conducted in accordance with the declaration of Helsinki and with the approval from the Ethics Committee of Xinjiang Obstetrics and Gynecology Hospital. Written informed consent was also obtained from all participants.

Adenomyosis was diagnosed by TVS exam according to the criteria described by Dueholm et al. [5]. Patients were excluded if they had any of the following conditions: hormonal contraception, chronic pelvic inflammatory disease, contraindications to progestins or contraindications to the use of an intrauterine contraceptive device. The symptom intensity was assessed by a 100-mm visual analogue scale (VAS), in which 0 indicated no pain and 100 indicated an unbearable pain. The severity of dysmenorrhea was graded as follows: a score of 1-50 was considered mild pain, 51-80 was considered moderate pain, and 81-100 was considered severe pain [7]. Once a woman met the inclusion criteria, she was requested to complete VAS scoring of dysmenorrhea. Her uterine volume, serum FSH, LH, E2, and PRL levels and menstrual pattern were recorded as the baseline variables before the LNG-IUS insertion. The uterine volume was calculated by using the formula

\[ \text{Uterine Volume} = \frac{\text{Height of Uterus} \times \text{Width of Uterus} \times \text{Length of Uterus}}{3} \]

A visual analogue scale (VAS) of dysmenorrhea, uterine volume and serum-levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and prolactin (PRL) were used to assess the efficacy of the treatment at baseline and at six and 12 months after the LNG-IUS. Serum-levels of FSH, LH, E2, and PRL were tested in pre-and post-insertion at six and at 12 months, respectively. Side-effects were recorded at every follow-up visit. Results: After six and 12 months of LNG-IUS insertion, dysmenorrhea was obviously alleviated, and the dysmenorrhea scores decreased to 2.6 from 0.6 (p < 0.05). The volume of uterus reduced six months after insertion and later, but without significant change (p > 0.05). After treatment of serum, in terms of FSH, LH, and E2 levels, compared with pre-insertion, there was no statistically significant difference (p > 0.01). However, the level of PRL markedly declined at six and 12 months after LNG-IUS. Conclusion: The LNG-IUS appears to be an effective method in alleviating dysmenorrhea associated with adenomyosis with little effect on ovarian function. It may be helpful to decrease the level of PRL in these patients.
for an ovoid: volume = D_1 \times D_2 \times D_3 \times 0.52. The LNG-IUS was then inserted into her uterine cavity during menses on cycle day 5-7 by a senior gynecologist. The LNG-IUS was composed of a T-shaped polyethylene core surrounded by a reservoir of 52 mg of LNG, which was delivered to the endometrium at a release rate of 20 mcg/day in a sustained fashion for five years.

The women underwent follow-up visits at three, six, and at 12 months after the LNG-IUS insertion. At each follow-up visit, a TVS was performed, a serum FSH, LH, E2, and PRL test was done and the VAS exams were performed by the same physician. Menstrual patterns before and after three, six, and 12 months of the LNG-IUS insertion were assessed and classified as normal, absent bleeding, infrequent bleeding, light regular bleeding, light prolonged regular bleeding, heavy regular bleeding or irregular bleeding [12]. Additionally, side-effects were also recorded at every visit. At the 12th month’s visit, the women were requested to rate her overall degree of satisfaction with the treatment as follows: very satisfied, satisfied, uncertain, dissatisfied or very dissatisfied [7].

In this study, data processing was conducted by means of SPSS 15.0 statistical analysis software, the data presenting normal distribution took the form of ± s, and those showing non-normal distribution adopted the form of M (P25, P75). T test was used to deal with those measurement data in line with the normal distribution, and Wilcoxon signed rank test, α = 0.05 was used to handle those data with non-normal distribution.

### Results

#### Pain Score

After the LNG-IUS insertion, dysmenorrhea, painful intercourse, chronic pelvic pain, dysmenorrhea VAS score, and sexual VRS pain score were significantly decreased (Tables 1 and 2). Among them, after inserting LNG-IUS for six months, there were 21 cases of disappearance of dysmenorrhea with patients (35.00%, 21/60), and 11 cases with sexual pain in patients with complete remission (68.75%, 11/16). At follow-up 12 months later, there were 17 cases with dysmenorrhea disappeared, corresponding to 28.33% (17/60).

### Measured results of reproductive hormone serum levels

Before LNG-IUS insertion and after LNG-IUS insertion for six and 12 months, in terms of serum FSH, LH, and E2 levels in 60 patients, there were no statistically significant differences (p > 0.05), whereas the PRL level was significantly lower than preoperative levels (p < 0.05, Tables 3 and 4).

### Renewal rate of patients after 12 months insertion

In the 12th month of follow-up, with the exception of one patient demanding to remove the ring, the other patients expressed their willingness to continue to use LNG-IUS, with a renewal rate of 98.33%. The former case patient was aged

#### Table 1 – The efficacy of LNG-IUS against pain M (P25, P75) after placement for six months.

<table>
<thead>
<tr>
<th>Time</th>
<th>Dysmenorrhea VAS score</th>
<th>Dysmenorrhea VRS score</th>
<th>Dyspareunia VAS score</th>
<th>Dyspareunia VRS score</th>
<th>Chronic pelvic pain VAS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before placing</td>
<td>8.5 (6.5,10.0)</td>
<td>2.0 (1,3.0)</td>
<td>0.0 (0,2.0)</td>
<td>0.0 (0,1.0)</td>
<td>0.0 (0,3.0)</td>
</tr>
<tr>
<td>6 months after placing</td>
<td>2.0 (0.0,3.0)</td>
<td>1.0 (0.0,1.0)</td>
<td>0.0 (0.0,0.0)</td>
<td>0.0 (0.0,0.0)</td>
<td>0.0 (0.0,0.0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.0002</td>
<td>0.003</td>
<td>0.0006</td>
<td>0.0008</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

#### Table 2 – The efficacy of LNG-IUS against pain M (P25, P75) after placement for 12 months.

<table>
<thead>
<tr>
<th>Time</th>
<th>Dysmenorrhea VAS score</th>
<th>Dysmenorrhea VRS score</th>
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<td>0.0 (0,2.0)</td>
<td>0.0 (0,1.0)</td>
<td>0.0 (0,3.0)</td>
</tr>
<tr>
<td>12 months after placing</td>
<td>0.0 (0,1.0)</td>
<td>0.0 (0,1.0)</td>
<td>0.0 (0,0.0)</td>
<td>0.0 (0,0.0)</td>
<td>0.0 (0,0.0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.0006</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

#### Table 3 – The efficacy of LNG-IUS on sex hormone levels after placement for six months regarding adenomyosis.

<table>
<thead>
<tr>
<th>Time</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>E2 (mmol/l)</th>
<th>PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before insertion</td>
<td>8.07±1.59</td>
<td>8.87±1.67</td>
<td>95.02±29.01</td>
<td>22.11±10.09</td>
</tr>
<tr>
<td>Follow-up after 6 months</td>
<td>7.84±1.44</td>
<td>8.55±1.52</td>
<td>103.07±24.92</td>
<td>18.11±7.20</td>
</tr>
<tr>
<td>t value</td>
<td>1.335</td>
<td>1.146</td>
<td>1.149</td>
<td>4.496</td>
</tr>
<tr>
<td>p value</td>
<td>0.249</td>
<td>0.061</td>
<td>0.471</td>
<td>0.001</td>
</tr>
</tbody>
</table>

#### Table 4 – The efficacy of LNG-IUS on sex hormone levels after placing for 12 months regarding adenomyosis.

<table>
<thead>
<tr>
<th>Time</th>
<th>FSH (mIU/ml)</th>
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</tr>
<tr>
<td>Follow-up after 12 months</td>
<td>7.67±1.23</td>
<td>8.43±1.17</td>
<td>104.35±24.90</td>
<td>19.45±5.73</td>
</tr>
<tr>
<td>t value</td>
<td>1.457</td>
<td>1.134</td>
<td>1.134</td>
<td>4.483</td>
</tr>
<tr>
<td>p value</td>
<td>0.240</td>
<td>0.057</td>
<td>0.396</td>
<td>0.001</td>
</tr>
</tbody>
</table>
46 years and demanded to remove the ring due to her menopause for seven months since the insertion of LNG-IUS. After the removal of the ring, her menopause continued with no recurrence of pain.

Discussion

This study shows promising effectiveness of using the LNG-IUS in alleviating adenomyosis-associated dysmenorrhea during a period of three years. The efficacy was observed throughout the three-year follow-up period, along with the reduction of uterine volume and serum PRL levels. The most remarkable changes in pain relief were observed at three and six months post-insertion, which were consistent with the results reported previously [11, 13]. Although the changes after six months were not as remarkable as those within the first six months, the authors must emphasize that the changes were constant and continuous, and the lowest VAS score was obtained at 36 months. In this study, the authors also found some side-effects reported by a small proportion of the women, which were the main reasons for premature removals. The discontinuation rate was 34% at three years, similar to that reported by a LNG-IUS study on endometriosis [14]. Albeit there were some side-effects, they were acceptable in comparison with the severe pain that those women previously experienced. The present study shows that 72.5% of women were very satisfied with the treatment at 12 months. Overall, the treatment has a high satisfaction rate. Since this was a conservative therapy, the authors could not prove the disappearance of the adenomyotic lesions, but the reduction of uterine volume and serum CA125 levels might reflect the shrinkage of the foci after the LNG-IUS insertion. Furthermore they also found shrinkage of adenomyoma and disappearance of some classic adenomyosis image by TVS in some women after the LNG-IUS insertion. In the present study, albeit there was a slight increase in uterine volume after 12 months of treatment, the difference was not significant compared with the volume at 12 months.

The mechanism of pain control action of the LNG-IUS in adenomyosis is unclear. It may correlate with serum levels of LNG or with the local concentration of LNG on the endometrium or with the combination of these two. The relatively high levels of serum LNG during the first months on the device (459.2 - 357.3 pg/ml) [15] may explain the dramatic improvements after the LNG-IUS insertion in a short period of time. The local mechanism may be the effect of high concentration of LNG on the eutopic endometrium, which results in glandular atrophy and stromal decidualization. A similar effect may also occur on the ectopic endometrium, which results in glandular atrophy and stromal decidualization. A similar effect may also occur on the ectopic endometrium, resulting in an endometrial inactivity to the estrogen in circulation via down-regulation of estrogen receptors [16]. In addition, the endometrial inactivity may decrease the production of prostaglandin I2, a substance that can cause pain and uterine contraction, resulting in pain relief [17]. Another local mechanism has been proposed: that the direct effect of the progestin on the junctional zone leads to a reduction in the invasion and progression of myometrial hypertrophy [13].

The significant value of the present study showed that after the use of LNG-IUS, the patients remained in ovulatory cycle, and it had little impact on ovarian function. Serum levels of reproductive hormones measured results show that in terms of FSH, LH, and E2, before and after the use of the LNG-IUS in patients with adenomyosis, there were no significant differences, however, the PRL level was significantly lower than the pre-placement of LNG-IUS. E2 levels in blood reflecting the ovarian function is the most direct evidence of impaired ovarian function, due to a reduction in the level of ovarian hormone secretion, the decline in performance for E2, but the rise of FSH and LH. The rise of FSH and LH is caused by the fact that the decrease in ovarian secretion of estrogen increases its role in the pituitary due to the negative feedback; while FSH and LH levels increase, it can inhibit follicle development and ovum development, so that ovarian function recesses and the generation of E2 further is reduced. As a result, monitoring the level of FSH and LH may be a more accurate assessment of ovarian function. Therefore, the blood FSH, LH, and E2 values of these 60 cases in this group were determined on the third day of menstrual cycle. The results showed that: for all patients, before and after placement of LNG-IUS for six and 12 months, there were no significant differences in FSH, LH, and E2 levels (p > 0.05), the placing of intrauterine LNG-IUS for the treatment of adenomyosis does not affect the patient’s ovarian function. After the use of LNG-IUS, the ovarian secretion of E2 remains normal and FSH and LH do not increase, without any effect upon reproductive health. The study also showed that with LNG-IUS placement, the PRL levels with patients can be reduced, or return to normal levels. The decline in PRL levels will be beneficial for patients to recover to normal pituitary gonadotropin secretion, to promote normal ovarian function and normal ovulation, and to regain normal corpus luteum function. Accordingly, patients are liable to return to normal menstruation and infertility patients have easier access to fertilization.

The relatively high expulsion rate in the present study may be due to enlargement of the patients’ uteri and distortion of the uterine cavities, resulting from adenomyosis and adenomyoma, which may increase the possibility of the device descending or expelling. Another possible reason for expulsion is heavy regular bleeding, which causes the device to be expelled. In the present study, the most common reason for premature removals was irregular bleeding followed by lower abdominal pain.

In the present study, the most common side-effect appeared to be weight gain. Since the authors did not have a control group and the mean age of the women was 36.8 ± 4.3 years, there was a possibility that this weight gain might be a reflection of changes that often occur in the general
community in this particular age group of women. Similar amounts of weight gain occur with the copper and LNG-IUS. Therefore, the authors cannot conclude that this weight gain is caused by the LNG-IUS. The incidence rate of other side-effects, such as breast tenderness, skin problems, and headaches, which may be related to the LNG in the circulation [18], is not higher than that of other medical treatments including danazol, continuous combined oral contraceptives and depot medroxyprogesterone [19]. The incidence of ovarian cyst formation is similar to that reported in a previous study [20]. Women with ovarian cysts were asymptomatic and had a high rate of spontaneous resolution. Despite the side-effects of this procedure, the women in this study showed a steady increase of satisfaction rate with a corresponding decrease of dissatisfaction rate along the 12 months treatment period, partly due to the alleviation of dysmenorrhea. In this study, women who had previously used analgesics such as NSAIDs and some Chinese traditional medicine no longer required their use or needed to use them only in very small doses after the LNG-IUS insertion.

This study has some limitations. First, it was not a multicenter, randomized, comparative study. Second, the diagnostic criterion of TVS for adenomyosis in the study had a sensitivity varying between 68% and 89% and a specificity between 65% and 99% [4, 21-23]. Third, overall satisfaction degree rating by the patients was used instead of a quality-of-life analysis to assess the overall effectiveness of the treatment.

In conclusion, the present study indicates that the LNG-IUS shows effectiveness in pain relief over a long period of time in women suffering from adenomyosis. Despite some side-effects, this treatment modality shows to be promising, with a high patient’s satisfaction rate on the increase over the 12 month treatment period. It also has little effect on ovarian function, as compared with other drugs. It also has a higher value of compliance and application and may be a valuable long-term alternative for the treatment of adenomyosis.

References


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Maternal soluble vascular cytoplasmic adhesion molecule-1 and fibronectin levels in early- and late-onset preeclamptic pregnancies

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¹Pozantı Government Hospital, Adana
²Department of Obstetrics and Gynecology, Faculty of Medicine, University of Cukurova, Adana (Turkey)

Summary

Objective: The purpose of this study was to investigate maternal plasma soluble vascular cytoplasmic adhesion molecule-1 (sVCAM-1) and fibronectin levels in the patients with early-onset preeclampsia (EOP) and late-onset preeclampsia (LOP) and also to determine whether different mechanisms are involved in these two forms of disorders. Material and Methods: The authors performed a case control study consisting of randomly selected 80 healthy pregnant women (group 1 = control group) and 80 preeclamptic women (group 2 = defined study group). Study group consisted of 43 patients with EOP and 37 patients with LOP. sVCAM-1 and fibronectin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) and the findings were compared between the groups. Results: The mean levels of sVCAM-1 and fibronectin were significantly higher in the LOP group than those in the normotensive group (p = 0.043 and 0.010 respectively). Markers were significantly different between the two hypertensive groups of pregnancy. The EOP group had a higher level of sVCAM-1 and fibronectin concentration than the LOP group (p = 0.01, for both markers). There was a positive correlation both between the values of plasma fibronectin and the systolic-diastolic blood pressure measurements (r:0.43 and 0.44, respectively), and between sVCAM-1 and the systolic/diastolic blood pressure measurements (r = 0.54 and 0.64, respectively). Conclusion: Increased plasma levels of fibronectin and sVCAM-1 were found in the preeclamptic patients, especially in those with early-onset preeclampsia. These markers might be related to the pathogenesis of different types of preeclampsia.

Key words: Soluble vascular cytoplasmic adhesion molecule-1; Fibronectin; Early- and late-onset preeclampsia.

Introduction

Preeclampsia is a heterogeneous human pregnancy syndrome that affects several organ systems [1]. It has been characterized by some investigators as two different diseases: early onset preeclampsia (EOP) and late onset preeclampsia (LOP) on the basis of gestational age [2-3]. EOP is usually defined as preeclampsia that develops before 34 weeks of gestation, while LOP develops at or after 34 weeks of gestation. Although the diagnostic criteria are the same in each of these phenotypic variants of preeclampsia, they are characterized by different clinical features and are associated with different maternal and fetal outcomes [2]. Gestational age at the onset of the disease is not considered as a criterion for the diagnosis or subclassification of preeclampsia [3].

Preeclampsia is proposed to occur in two stages. Stage 1 comprises reduced placental perfusion, which is postulated as the root cause that leads to Stage 2, namely the maternal syndrome [4]. It is believed that placental ischaemia during Stage 1 may lead to placental production factors, one of which is cytokines that cause the activation of adhesion molecules [5, 6]. The cell adhesion molecules play a role in leukocyte-endothelial interaction and diapedesis [5, 6]. The basic processes of leukocytes are to phagocyte the useful factors and to produce toxic factors. These toxic factors (elastase, myeloperoxidase) injure the endothelium [6, 7] and dysfunctional endothelium leads to the clinical syndrome of hypertension and proteinuria [8].

Adhesion molecules are divided into groups according to their structures: selectins, integrins, cadherins, and members of the immunoglobulin gene superfamily. Besides these groups, there are some molecules performing the same functions as adhesion molecules like fibronectin [9, 10]. sVCAM-1 is a cell adhesion molecule and a member of the immunoglobulin superfamily. sVCAM-1 is important for recruiting leukocytes to the sites of inflammation because it mediates the adhesion of lymphocytes, monocytes, and eosinophils to endothelium [11].

Increased levels of sVCAM-1 and fibronectin in the patients with preeclampsia could be indicative of endothelial cell activation and the soluble adhesion molecules in plasma should reflect the concentration of membrane-bound adhesion molecules on the endothelium. Thus, in this study the authors aimed to evaluate the role of sVCAM-1 and fibronectin in the patients with EOP and LOP.

Material and Methods

This study was conducted as a case-control study in prospective cross-sectional cohort type between March 2008 and March 2009. Eighty normotensive patients indicated as healthy pregnant according to the results of the examinations were included in this...
Table 1. — Clinical characteristics of study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>EOP (n=43)</th>
<th>LOP (n=37)</th>
<th>Normotensive controls (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>29.39±5.91</td>
<td>30.12±1.92</td>
<td>28.00±5.84</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1.42±1.38</td>
<td>1.43±1.56</td>
<td>0.93±1.18</td>
</tr>
<tr>
<td>GA</td>
<td>25.49±3.72</td>
<td>34.12±1.12*</td>
<td>37.65±2.50</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1819.52±800.4*</td>
<td>2467.44±996.0*</td>
<td>3209.38±648.47</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>160.12±15.12*</td>
<td>150.90±13.01</td>
<td>110.87±10.34</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>110.14±14.13**</td>
<td>102.60±10.81*</td>
<td>67.87±8.22</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation (SD).

*Significant difference in comparison with normotensive and late-onset preeclamptic groups (p < 0.05).

**Significant difference in comparison with early-onset and late-onset preeclamptic groups (p < 0.05).

GA: Gestational age at delivery; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

study as control group (group 1) and 80 patients with preeclampsia who were hospitalized in the Obstetrics Clinic of Medical Faculty at Çukurova University were included as study group (group 2). Preeclamptic patients were divided into two groups according to the onset of the preeclampsia: early-onset preeclamptics (< 34 weeks gestation) (n=43) and LOPs (≥ 34 weeks gestation) (n=37). At the stage of sampling for blood analysis, medical consent was taken from each case included in the study in accordance with the ethical issues in the Declaration of Helsinki. Furthermore, ethical approval of the Ethics Committee of Cukurova University was obtained. The women who gave written informed consent were recruited in this study. The cases of the study and control groups were chosen from the patients whose pregnancies were in the 20th and 41st weeks. While indicating gestational age, the dates of the last menstruation and obstetrical ultrasonographic examination were taken into consideration. All of the cases were chosen from the pregnancies who had no histories about hypertension or any diseases affecting energy metabolism and drug use, smoking, singleton, diabetes, and any autoimmune systemic diseases. Patients whose tension arterial rates were over 140/90 mmHg in two measuring six hours apart and protein losses were over 300 mg/l in urine in 24 hours or who had one positive proteinuria in spot urine were accepted as preeclamptic. Control group consisted of the pregnancies who had no histories about systemic diseases and drug use, smoking, normotension, and proteinuria. Demographic data and histories, complete blood count, and complete urine analysis were checked for all pregnancies included in the study. Also, serum blood urea nitrogen, creatinine, AST, ALT, and LDH values were evaluated. In addition to this, the values of total protein were controlled by collecting a 24-hour urine sample from the preeclamptic group. Venous blood samples of all pregnancies from antecubital zone were analyzed. After blood samples taken from fibronectin and sVCAM-1 antecubital vein under sterile conditions were centrifuged for five minutes in 3,500 cycles, their plasma was taken and kept in -70ºC in laboratory until analyses were begun. Serum levels of fibronectin and sVCAM-1 were measured by commercial enzyme-linked immunosorbent assay (ELISA) assay according to the manufacturer’s instructions.

All values were expressed as means ± SD (standard error of mean). Statistical tests were performed using SPSS (Statistical Package for Social Sciences) version 15.0. Variations over statistically significant results and rates between the groups were indicated by using Whitney U, chi-square, and Spearman correlation tests. A p-value of < 0.05 was considered statistically significant.

Table 2. — Biochemical parameters of study and control groups.

<table>
<thead>
<tr>
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<th>Normotensive controls (n=80)</th>
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<tbody>
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<td>sVCAM-I (ng/ml)</td>
<td>87.27±38.11**</td>
<td>50.59±33.22*</td>
<td>45.03±33.91</td>
</tr>
<tr>
<td>Fibronectin (ng/ml)</td>
<td>30.22±8.29**</td>
<td>20.33±5.11*</td>
<td>12.97±10.83</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>70.44±30.33**</td>
<td>34.59±38.72*</td>
<td>18.32±5.37</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>76.33±23.22**</td>
<td>33.71±31.75*</td>
<td>18.16±5.05</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>10.81±3.21</td>
<td>10.12±2.33</td>
<td>10.63±3.21</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.86±0.40</td>
<td>0.78±0.80</td>
<td>0.77±0.39</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>34.45±4.67</td>
<td>33.34±4.34</td>
<td>32.94±4.25</td>
</tr>
</tbody>
</table>

*Significant difference in comparison with normotensive and late-onset preeclamptic groups (p < 0.05).

**Significant difference in comparison with early-onset and late-onset preeclamptic groups (p < 0.05).

Results

A significant variation was not indicated in terms of age and gravid between preeclamptic and normotensive pregnancies. Gestational weeks at delivery and birth weight of the newborns were significantly lower in the LOP group than in the normotensive group and were significantly lower in the EOP group than in the LOP group (p < 0.05). As expected, blood pressure measurements were significantly higher in the LOP group than in the normotensive group (p < 0.05) and blood pressure measurements were significantly higher in the EOP group than in the LOP group (p < 0.05, Table 1).

The serum concentrations of sVCAM-1, fibronectin, AST, and ALT were significantly higher in the LOP group than in the normotensive group (p = 0.043, 0.010, 0.010, and 0.010, respectively) and also these parameters were significantly higher in the EOP group than in the LOP group (p = 0.010, for all variables). There were no significant differences in the mean values of the serum creatinine, hematocrit, and BUN among all groups (p > 0.05, Table 2). The serum fibronectin concentrations were significantly higher in the EOP group (30.22 ± 8.29 ng/ml) than in the LOP group (20.33 ± 5.11 ng/ml).
ng/ml) (p = 0.01, Figure 1). The serum sVCAM-1 concentrations (87.27 ± 38.11 ng/ml) were significantly higher in the early-onset pre eclamptic group than in the LOP group (50.59 ± 33.22 ng/ml) (p = 0.01, Figure 2).

In the preeclamptic patients, the authors determined a positive correlation between plasma fibronectin values and the systolic and diastolic blood pressure measurements (r = 0.43 and 0.44, respectively) and they also found a positive correlation between sVCAM-1 and the systolic and diastolic blood pressure measurements (r = 0.54 and 0.64, respectively, Table 3). It was found that neonatal mortality and morbidity were significantly higher in the EOP group than in the LOP group (p = 0.002, Table 4).

**Discussion**

Despite the still unexplained pathogenesis, preeclampsia is thought to be the result of generalized endothelial dysfunction [12]. Increased levels of cell adhesion molecules, which are the excessive production of underperfused placentas, are believed to be indicators of endothelial dysfunction in preeclampsia [13]. In the present study, serum concentrations of sVCAM-1 were significantly higher in the preeclamptic group, especially EOP group. Lyall et al. are the first to show that sVCAM-1 is elevated in the serum of preeclampsia patients [7]. There are numerous studies that show an increase of adhesion molecules in preeclamptic patients [14-17]. Few studies have investigated the relationship between the markers of endothelial dysfunction and the severity of preeclampsia or pregnancy outcomes. Djurovic et al. published that maternal concentrations of sVCAM-1 were significantly elevated in both mild and severe preeclampsia and also in preeclampsia with SGA infants [18]. In a study by Shim-Young Kim et al., it was revealed that sVCAM-1 was statically meaningful in estimating preeclampsia progression [19].

Measurement of plasma fibronectin level, which is the indicator of endothelium cell damage in the recognition of preeclampsia, showed an increase in the fibronectin levels in the preeclampsia cases [20-23]. In the present study, serum concentrations of fibronectin were significantly higher in the LOP group than in the normotensive group and they were also significantly higher in the EOP group than in the LOP group. In a study conducted by Power et al., the effect of fibronectin level on adverse pregnancy outcome was examined in the preeclampsia patients and it was seen that elevated fibronectin was prevalent among the women with preeclampsia and the women were identified to be at increased risk for preterm delivery and SGA [24]. Fibronectin and sVCAM-1 levels were observed to be significantly high in direct proportion to the severity and the onset of the diseases. The present results demonstrated that the evidence of endothelial dysfunction in the women with EOP was associated with increased risk of adverse pregnancy outcomes.

Several investigators have proposed that EOP and LOP may have different pathophysiology and that these two phenotypes should be studied individually [3, 25, 26]. EOP is associated with greater perinatal and maternal mortality and morbidity than late-onset disease [27-29]. The present authors found that neonatal mortality and morbidity were significantly higher in the EOP than in the LOP group (p = 0.002). As expected, there was a positive correlation between plasma fibronectin and sVCAM-1 and the blood pressures in this study, which may be attributed to the hypertension-inducing effects of adhesion molecules.

Govender et al. investigated the role of angiogenic, antiangiogenic and vasoactive factors in the black South African women with EOP and LOP. They suggested that the excess of serum sFlt-1 and reduced VEGF and PI GF levels favored an anti-angiogenic state and endothelial dys-

| Table 3. — Plasma fibronectin and sVCAM-1 blood pressure correlation in study group. |
|---------------------------------|-----------------|-----------------|
|                                 | Systolic blood pressure | Diastolic blood pressure |
|                                 | r     | p      | r     | p      |
| Fibronectin                     | 0.43  | 0.0001 | 0.44  | 0.0001 |
| sVCAM-1                         | 0.54  | 0.0001 | 0.64  | 0.0001 |

r: correlation coefficient

<table>
<thead>
<tr>
<th>Table 4. — The comparison of neonatal morbidity and mortality between the early-onset and late-onset preeclamptic groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-onset preeclampsia (n=43)</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Neonatal mortality</td>
</tr>
<tr>
<td>Neonatal morbidity</td>
</tr>
<tr>
<td>Healthy infant</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Pearson Chi-Square Test (p = 0.002)
function leading to preeclampsia and that the aetiology and pathogenesis of EOP and LOP differ [30]. Groten et al. investigated the expression of VE-cadherin and vascular endothelial growth factor receptor-2 (VEGFR2) in preeclampsia. The findings of their study lead the present authors to conclude that the aetiology and pathogenesis of EOP and LOP are to some extent different [31].

The present study is the first to demonstrate that the concentrations of fibronectin and sVCAM-1 are higher in the EOP group than in the LOP group. These different values between EOP and LOP seem to support the opinion that the etiology and pathogenesis of preeclampsia. The findings of their study lead the present authors to conclude that the etiology and pathogenesis of preeclampsia: does it indicate the mechanism of leucocyt activation?" Br. J. Obstet. Gynaecol., 1994, 101, 143.


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Can combination of Day 3 and Day 5 embryo morphology be useful to predict pregnancy in in-vitro fertilization cycles?

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Summary

Objective: To determine whether embryos having all top qualified both on Day 3 and Day 5 have higher pregnancy rates than the others. Materials and Methods: The study included 143 consecutive cycles were recruited in which Day 5 embryo transfer was available. Cleavage stage embryos were graded according to 1 to 4 scoring system, based on fragmentation, cell symmetry, and blastomere number. Among cleavage stage embryos, Grade 1 and Grade 2a/2b were further stratified as ‘top quality’ embryos to be transferred, others were defined as control group. Blastocyst stage embryos were graded from 1 to 6 according to intracellular mass (ICM) and trophoderm (TE). Day 5 fresh embryo transfer was performed in all cases using soft catheter. Positive pregnancy test was accepted when serum beta-human chorionic gonadotrophin (ß-hCG) exceeded 20 mIU/ml. Results: On the cleavage stage, top quality embryo was available in 47 of 143 (32.9%) cases. Of the 47 embryos, the number of cases reaching any Grade 4, 3 quality, and early blastocyst on Day 5 were 22 (46.8%), 15 (31.9%), and 10 (21.3%). The respective figures on the control group (n=96) were 33 (34.4%), 37 (38.5%), and 26 (27.1%) (p > 0.05). The pregnancy rates were also similar. Conclusion: All top qualified embryos both on Day 3 and 5 did not reveal higher pregnancy rate than the others.

Key words: Embryo morphology; Blastocyst; In vitro fertilization.

Introduction

Since the first infant was achieved via in-vitro fertilization (IVF) in 1978 [1], numerous researches have been made for better understanding the development of human embryo. In clinical practice, regarding the day of transfer, either cleavage-stage embryo or blastocyst might be preferred according to patient characteristics, number of available embryos, and local legislation. However, irrespective from the day of transfer, assigning the one to be transferred within the available embryos mainly depends on cell number and morphology.

Nowadays, single embryo transfer revealing singleton pregnancy and a healthy infant is the most desired outcome in IVF cycles. Therefore, selecting the single embryo having the highest chance of implantation is crucial to prevent multiple pregnancies while keeping the success rate at a reasonable level. Although there is enough evidence addressing improved pregnancy rate with blastocyst transfer compared with cleavage stage [2], there is paucity of data whether the former quality on Day 3 affects the morphology and implantation potential of an embryo on Day 5. Nevertheless, one may assume that, the implantation potential might present diversity within blastocysts having similar scores for morphology on Day 5 but having different scores on Day 3.

In the current study, primarily, the authors sought to determine whether embryos having all top qualified according to morphological assessment both on Day 3 and Day 5 have higher pregnancy rates than the remaining. Secondly, the authors aimed to observe the natural way of cleavage-stage embryos according to blastocyst morphology on Day 5.

Materials and Methods

Between March 2010 and September 2012, a total of 143 consecutive cycles were recruited retrospectively in which Day 5 embryo transfer was available. Frozen-thawed cycles and women that embryo transfer was available. Frozen-thawed cycles and women that were transferred on Day 3 were excluded from the final analysis.

Controlled ovarian hyperstimulation

All patients underwent controlled ovarian hyperstimulation consisting of either luteal long gonadotrophin-releasing hormone (GnRH) agonist or fixed GnRH antagonist protocols with gonadotrophin. The starting dose of gonadotrophin was determined based on female age, antral follicle count at baseline transvaginal ultrasonography, body mass index (BMI), and previous ovarian response, if available. Ovarian response was monitored with frequent serum estradiol measurements and transvaginal ultrasonography, as described previously [3]. The criterion for human chorionic gonadotrophin (hCG) administration was the presence of two or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound-guided puncture of follicles 36 hours after hCG administration. Embryo transfer was performed using soft catheter under transabdominal ultrasound guidance. The luteal phase was supported by daily vaginal progesterone suppositories starting one day after oocyte pick-up.

Embryo and blastocyst grading

All oocytes are fertilized using intracytoplasmic sperm injection (ICSI). For the procedure of ICSI, the most morphologically normal motile spermatozoa were identified. Where all spermatozoa
had morphological defects, spermatozoa with fully developed tails and grossly normal heads were injected. Vitality testing of immotile spermatozoa was not performed. The presence of fertilization was evaluated by examining oocytes 12–17 hours after injection for the presence of distinct two pronuclei and two polar bodies.

Cleavage stage embryos were graded according to a 1 to 4 scoring system, which was based on fragmentation, cell symmetry, and number of blastomere as referred by Hardarson et al. [4]. The scoring system was as follows: Grade 1 embryo: no fragmentation with equal size homogenous blastomeres, Grade 2a embryo: <10% fragmentation with equal size homogenous blastomeres, Grade 2b embryo: unequal size blastomeres with no fragmentation, Grade 2ab embryo: unequal size blastomeres with <20% fragmentation, Grade 3 embryo: equal or unequal size blastomeres with 30-50% fragmentation, Grade 4 embryo: >50% fragmentation. Among cleavage stage embryos, whereas Grade 1 and Grade 2a / 2b were further stratified as ‘top quality’ embryos to be transferred, others were defined as control group.

Blastocyst stage embryos were graded from 1 to 6 according to intracellular mass (ICM) and trophectoderm (TE), as reported by Gardner and Schoolcraft [5]. In this grading system, grades refer to: Grade 1: early blastocyst (blastocoel being less than half the volume of embryo), Grade 2: blastocyst (blastocoel being greater than half the volume of embryo), Grade 3: full blastocyst (blastocoel completely fills the embryo), Grade 4: expanded blastocyst (blastocoel volume is larger than that of early embryo and zona is thinner). ICM is graded as A, B, and C. Whereas A refers to tightly packed and many cells, B describes loosely grouped and several cells. C refers to very few cells. TE is also graded as A, B, and C. Whereas A refers to many cells forming cohesive epithelium, B signifies few cells forming loose epithelium. C includes TE with very few large cells. Women were further categorized according to the quality of transferred embryos on Day 5 as 4AA/4AB; 4BB/4BA; 3AA/3AB; 3BB/3CC, and early blastocyst. Positive pregnancy test was accepted when serum b-hCG exceeded 20 mIU/ml. Ethical approval was available from Hacettepe University.

Statistical analysis

Statistical analysis of the results was performed using statistical package for the social sciences software (SPSS version 16.0). Clinical characteristics are compared using independent samples t test. The \( \chi^2 \) test and Fisher’s exact test were used to analyse variables in the form of frequency tables. A \( p \) value < 0.05 was considered statistically significant.

Results

On the cleavage stage, top quality embryo was available in 47 of 143 (32.9%) cases. The mean female age, BMI, duration of infertility, and number of oocytes retrieved were comparable among patients having top quality embryo on Day 3 and controls (Table 1).

Table 1. — Characteristics of the patients according to embryo quality on Day 3.

<table>
<thead>
<tr>
<th>Female age (years)</th>
<th>BMI (kg/m²)</th>
<th>Duration of infertility (months)</th>
<th>No. of oocytes retrieved</th>
<th>Number of cells of day 3 embryos</th>
<th>Fertilization rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.3 ± 3.3</td>
<td>24.3 ± 3.7</td>
<td>67.6 ± 40.5</td>
<td>13.2 ± 4.5</td>
<td>7.8 ± 0.7</td>
<td>83</td>
</tr>
<tr>
<td>28.9 ± 3.8</td>
<td>25.3 ± 4.3</td>
<td>58.6 ± 43.3</td>
<td>12.2 ± 6.0</td>
<td>7.7 ± 1.1</td>
<td>82</td>
</tr>
</tbody>
</table>

Data given as mean ± standard deviation, unless stated otherwise. All comparisons are non-significant.

Of the 47 embryos, the number of cases reaching any Grade 4, 3 quality, and early blastocyst on Day 5 were 22 (46.8%), 15 (31.9%), and 10 (21.3%). The respective figures in the control group was 33 (34.4%), 37 (38.5%), and 26 (27.1%) (\( p > 0.05 \)). On Day 5, the pregnancy rate per embryo transfer was highest when 4AA/4AB was available when compared with 3AA/3AB and early blastocyst arms (Table 2). However, success rate did not present significance when matched according to the former quality of embryos on Day 3 (Table 2).

To investigate the natural course of embryo morphology, the scores on Days 3 and 5 are given in Table 2. However pregnancy rates were similar when embryos were categorized according to the quality both on Days 3 and 5.

Discussion

To the best of the authors’ knowledge, there is no study that has examined the pregnancy outcome by combining Days 3 and 5 embryo morphology. Therefore the aim of this study was to search whether the pregnancy rate could be increased by selecting the best embryo to be transferred by combining scores on both days. However, the authors failed to present any advantage of combination embryo grades in means of pregnancy rate. Of note, a top qualified embryo on Day 3 did not reveal a clear course for the quality on the stage of blastocyst. Nevertheless, only 22 of 47 top qualified embryos (46.8%) reached a Grade 4 embryo on Day 5. Similarly, of the 35 blastocyst assigned to Grade 4, 17 of them (48.6) had been scored top qualified on Day 3 and pregnancy rate did not differ; either they were all top qualified or not (Table 2). Therefore, once an embryo

Table 2. — Pregnancy rates according to morphology on Days 3 and 5.

<table>
<thead>
<tr>
<th>Day 3 embryo quality (n, %)</th>
<th>Day 5 embryo quality (n, %)</th>
<th>Early blastocyst</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>4AA / 4AB</td>
<td>3AA / 3AB</td>
<td>3BB / 3CC</td>
<td></td>
</tr>
<tr>
<td>10/17 (58.8)</td>
<td>4/10 (40.0)</td>
<td>4/10 (40.0)</td>
<td>22/47 (46.8)</td>
</tr>
<tr>
<td>2/5 (40.0)</td>
<td>2/5 (40.0)</td>
<td>9/17 (52.9)</td>
<td>41/96 (42.7)</td>
</tr>
<tr>
<td>8/15 (53.3)</td>
<td>8/26 (30.8)</td>
<td>11/22 (50.0)</td>
<td>63/143 (44.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls (n=96)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>22/35 (62.9)</td>
<td>8/30 (40.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/20 (50.0)</td>
<td>11/22 (50.0)</td>
<td></td>
</tr>
</tbody>
</table>

* \( p = 0.006 \) and \( \beta = 0.018 \).
Can combination of Day 3 and Day 5 embryo morphology be useful to predict pregnancy in in-vitro fertilization cycles?

Can combination of Day 3 and Day 5 embryo morphology be useful to predict pregnancy in in-vitro fertilization cycles? 687

reached blastocyst stage, its preceding quality on Day 3 seemed to have no impact on the pregnancy rate.

Due to the inherent maternal and neonatal risks of multiple gestations, there is a trend towards transfer of a single blastocyst on day 5 in the field of assisted reproductive technology (ART) in order to minimize multiple pregnancies. According to current legislation in Turkey after March 2010, it became obligatory to transfer one embryo in the first two cycles of IVF in patients <35 years of age and only two embryos can be transferred at maximum after the age of 35, whatever the cause of infertility is. When transferring a single embryo, finding the best embryo having the highest potential of implantation is crucial. On the cleavage stage, morphologic assessment based on cell symmetry and fragmentation is widely preferred [6] since non-morphologic tools called omics technologies (genomics, transcriptomics, proteomics, and metabolomics) has been a challenging but promising task. Despite the developments in omics, the knowledge of the protein secretome of preimplantation embryos remains limited. The combined effects of limited template, low protein expression, and lack of sensitivity of current proteomics platforms remain main hurdles [7]. To date there is no non-invasive platform, including non-invasive proteomics that has proven to be of true clinical predictive value or been examined in prospective randomized control trials to be better than current morphology based selections methods. As for metabolomics, several studies have suggested the presence of metabolic differences between embryos with different reproductive potential [8]. However the application of these technologies to a clinical setting has remained limited for a variety of reasons. Many of these technologies are expensive, require dedicated equipment and technical staff, and frequently do not produce results quick enough to allow information to be used clinically in the limited window of time acceptable for embryo transfer. Moreover, none of these technologies has ever been validated using culture media evaluated in a blinded fashion and shown to correlate with the implantation potential of embryos that have been transferred. There appears to be a clear relationship between morphologic grading of cleavage stage embryos and their implantation potential [9]. Excellent and good quality embryos (grade 1 and 2) have a higher chance of implantation compared to fair and bad quality embryos (Grades 3 and 4). With regards to blastocyst embryos, grading is based on morphology of ICM and TE as well as expansion of the blastocyst cavity [10]. Blastocyst quality has been shown to be clearly associated with implantation and pregnancy [11]; with good quality blastocysts yielding very high implantation rates and hatching blastocysts having the highest. The present data also show that highest quality of embryos at blastocyst stage have the highest pregnancy rates.

The relationship between cleavage-stage embryo quality and blastocyst quality was studied by Rjinders and Jansen [12]. They concluded that the predictive value of day 3 embryo morphology regarding subsequent blastocyst formation is limited because only 51% of the embryos that would have been preselected for transfer on day 3 could reach blastocysts on day 5. Although 47% of the good quality embryos reached the blastocyst stage, only 21% of the poor quality embryos did so on day 5 [11].

Prolonged in vitro culture and transfer at the blastocyst stage of poor quality cleavage-stage embryos appears to increase implantation rates. Balaban et al. reported that despite fewer embryos being available for transfer on day 5, pregnancy rates are not compromised when compared with day 3 transfers [11]. But at the time this study was conducted, multiple embryo transfers were allowed. Their mean number of embryos transferred was 5.2 at day 3 and 2.4 at blastocyst stage. The present authors transferred single embryo and found pregnancy rates of blastocyst arising from both good and poor quality embryos to be same.

In a recent study, Mackenna et al. searched sibling embryo blastocyst development as a prognostic factor for the outcome of day 3 embryo transfer [13]. They stated that development of sibling embryos to blastocyst is a prognostic factor for the outcome of the cycle in which transfer is performed at day 3 and provides valuable information about the prognosis of the subsequent cycle. Although they graded the embryos transferred at day 3, no data has been given about the quality of blastocysts developed from sibling embryos. According to Mackenna et al. sibling embryo blastocyst development is important whereas the present authors only included embryos reaching blastocyst stage in this study, and according to these results, once an embryo reaches blastocyst stage, its respective day 3 quality possibly has no importance.

Overall, analysis of embryo morphology and using various grading systems may aid in the selection of embryos that may have the highest potential for implantation. However, they remain insufficient to predict the successive pregnancy. Non-invasive methods that assess the embryo culture media like proteomics and metabolomics may be combined with the embryo grading systems in the near future to further select the best embryo.

Limitations of the present study are: firstly it is not a prospective randomized study that is several biases of a retrospective study might have been seen. Secondly, the authors did not search for the rate at which poor or good quality embryos reached blastocyst stage as would have been done in a prospective study, since it was previously reported that embryos reaching blastocyst stage might have increased pregnancy rates. Instead the authors evaluated their respective day 3 embryo qualities of embryos reaching blastocyst stage. This would not be applicable in patients having few number of embryos which would be transferred at day 3, whether being poor or good quality due to the risk of halting embryo development before reaching day 5. Thirdly, the authors did not have any Grade 2 blastocysts transferred that might have possible effects on the results.
In conclusion, embryos on Day 3 present no clear course regarding the morphology at the blastocyst stage. All top qualified embryos both on Day 3 and 5 do not reveal higher pregnancy rates than the others. Those findings once again suggest that morphological-only assessment is not competence enough for the prediction of pregnancy in IVF.

The question of which embryo to be selected in the situation where multiple embryos of the same patient reach the same grade of blastocyst stage may be subject of future studies.

References


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Introduction
Pelvic inflammation is a rare but serious complication that arises following oocyte pickup in an in vitro fertilization (IVF) program [1]. Pelvic inflammation is reported to occur in approximately 0.5% of the infertile patients undergoing oocyte pickup, even with prophylactic antibiotics administration [2]. The major cause of oocyte pickup-associated pelvic inflammation (OPU-PI) is considered to be iatrogenic dissemination of vaginal microorganisms into abdominal cavity in the process of follicular needle aspiration [3].

Early studies demonstrated saline douching immediately before OPU as a safe and effective vaginal preparation procedure to wash out the vaginal bacterial flora without compromising the reproductive outcome [4]. However, accumulating studies report that vaginal saline douching is insufficient to prevent moderate-to-severe OPU-PI including peritonitis and ovarian abscess [5-7]. The aim of this study was to compare the preventive effects on OPU-PI and the reproductive outcome between the two groups using Fisher exact test. The parameters for clinical demographics were statistically compared using Student’s t test.

Materials and Methods
From April 2007 to March 2009, 956 infertile patients underwent OPU in the present IVF center. The vulva was rinsed with sterilized saline solution and the vagina was douched similarly (approximately 100 ml) immediately before OPU. From April 2009 to March 2011, vaginal preparation procedure was switched to a combination of povidone iodine disinfection and following saline douching for 1,216 infertile patients undergoing OPU [8]. The vulva was rinsed with sterilized saline solution and the vagina was disinfected with aqueous povidone iodine (approximately 50 ml) and then douched with saline solution (approximately 100 ml) immediately before OPU. All patients underwent prophylactic antibiotics intravenous administration of fosfomycin (one g, drip infusion, starting before OPU), along with postoperative two-day oral administration of clarithromycin (400 mg/day) or cefcapene pivoxil hydrochloride (300 mg/day). IVF was performed as described previously [9].

Results
In the vaginal saline douching group, four out of 956 patients (0.42%) were diagnosed with OPU-PI due to fever, abdominal pain, leukocytosis, and elevated serum C-reactive protein (Table 1). The onset of OPU-PI ranged from the day 1 to day 40 following OPU. All these patients with OPU-PI had had a past history and/or present illness of endometriosis. In three patients with ovarian endometrioma, OPU-PI occurred despite that the authors carefully avoided puncture and aspiration of the cysts. Antibiotics therapy alone was effective in two patients, whereas the other two patients required surgical treatments for ovarian en-
dometrioma. Two out of four patients had a successful pregnancy within six months of OPU and live birth.

In the vaginal povidone iodine disinfection/saline douching group, there were no patients who developed OPU-PI. Vaginal preparation with povidone iodine disinfection and saline douching was significantly effective in prevention of OPU-PI over that with saline douching alone ($p = 0.016$). Meanwhile, there were no significant differences in the age, body mass index, gravidity, parity, fertilization rate, morphologically good embryo acquisition rate, clinical pregnancy rate, and ongoing pregnancy rate between the two groups ($p > 0.23$).

Discussion

One of the critical risk factors for OPU-PI is the presence of endometriosis, particularly when the ovarian endometrioma is unintentionally punctured and the content fluid leaked into abdominal cavity [3-5]. In consistent with these reports, the present authors confirmed the close link between endometriosis and OPU-PI, as all patients had its past history and/or present illness. The onset of OPU-PI ranged from the day 1 to day 40 following OPU.

This large cohort study demonstrated that vaginal povidone iodine disinfection immediately before OPU is an effective tool regarding prevention of PI. There has been a concern that povidone iodine acts as a toxic agent for oocytes when it was used as vaginal disinfectant [4]. The present findings, however, support the previous report showing that vaginal preparation with a combination of povidone iodine disinfection and subsequent saline douching did not reduce the fertilization and implantation rate in the IVF cycle [6]. These findings suggest that the good rinse with saline may be important to block the intraperitoneal dissemination of povidone iodine and maintain the integrity of the oocytes aspirated.

Following adoption of vaginal povidone iodine disinfection and saline douching from April 2009 onwards, the authors have not seen the cases with OPU-PI. This procedure is simple, but cheap and effective enough to prevent OPU-PI without spoiling the oocyte quality.

References


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Elevated tissue levels of tumor necrosis factor-α in vulvar vestibulitis syndrome

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Summary

The purpose of this study was to compare levels of inflammatory cytokines, namely TNF-α, IL-1β, and IL-1 receptor in women with vulvar vestibulitis syndrome (VVS) relative to levels in controls. The authors hypothesized that tissue concentrations of inflammatory cytokines would be elevated significantly in women with VVB compared to pain-free controls. The study population consisted of 15 women with strictly defined VVB in reproductive age and 13 age-matched women with no history of vulvodynia. For TNF-α, positive staining was observed in 40% of the samples from the study group and in 7.7% of the samples from the control group. The difference between the groups was statistically significant ($p < 0.05$). In conclusion, a limitation of the present study was the relatively small sample size. However, the authors’ intention was simply to propose that the local inflammation may be mediated by cytokines as TNF-α may rather than trying to single out a pathogenesis of VVS. The authors’ findings of elevated TNF-α may suggest new therapeutic alternatives for VVS, as inhibiting cytokine synthesis or antagonism of the cytokine receptor.

Key words: TNF-α; IL-1β; IL-1 receptor; Vulvar vestibulitis syndrome.

Introduction

Vulvar vestibulitis syndrome (VVS) was first described nearly a century ago [1] and is currently one of the most frequent causes of coital pain in women within premenopausal period [2]. However, gynecologists have only recently begun to pay more clinical attention to this intriguing syndrome. Vulvar vestibulitis is a chronic clinical syndrome characterized by severe pain on vestibular touch or attempted vaginal entry, tenderness to a cotton-swab palpation of the vestibular area, and physical findings confined to vestibular erythema [3]. It has received increasing attention from a number of multi-disciplinary researchers, including gynecologists, dermatologists, urologists, psychiatrists, and psychologists. The syndrome is regarded as one of the subtypes of vulvar pain and dyspareunia [2, 4]. In addition, the suspected prevalence seems to be increasing, with reported rates of <15% [3, 5, 6].

The etiology VVS remains unknown and is probably multifactorial. However, the chronic inflammation might be related to a decreased ability to cease the inflammatory response due to prolonged induction of pro-inflammatory cytokines. Some researchers propose a neuropathic etiology for VVS, because thresholds to thermal and mechanical stimuli are lowered in VVS patients like in other neuropathic pain syndromes [7-9].

Recent studies propose that pro-inflammatory cytokines, like interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), may have a role in the pathogenesis of neuropathic pain [10, 11]. Furthermore, some studies suggest that these factors may aggravate inflammatory diseases [12-14].

The purpose of this study was to compare levels of inflammatory cytokines, namely TNF-α, IL-1β and IL-1 receptor in women with VVB relative to levels in controls. The authors hypothesized that tissue concentrations of inflammatory cytokines would be elevated significantly in women with VVB compared to pain-free controls.

Materials and Methods

Study population

The study population consisted of 15 women with strictly defined VVB in reproductive age and 13 age-matched women with no history of vulvodynia. The study was carried out in the gynecology clinic of Istanbul University School of Medicine.

Strict inclusion criteria were: (1) ≥ one year of vulvar burning with insertional dyspareunia or pain with tampon insertion; (2) tenderness to light touch, limited to the vulvar vestibule between Hart’s line and the hymeneal tissue (cotton-tip applicator was used to record a visual analog scale of 1 to 10); (3) ≤ six-month medical therapy did not relieve the pain.

Exclusion criteria were: (1) usage of antibiotics or immunosuppressive medications in last 30 days; (2) a clinically apparent microbial infection; (3) any other neuropathology.

All perineoplasty procedures were performed by one of the authors (S.E.A) and vulvar vestibule was confirmed by a second independent examiner.

The controls were women that underwent rectocele repair (posterior colporrhaphy) in the last six months who were pain-free and had a normal neurologic exam.

This investigation was approved by the Ethics Committee of Istanbul University and written consent from the subjects were obtained prior to the initiation of the study.

Immunohistochemistry evaluation

Expression of TNF-α, IL-1β and IL-1 receptor in tissue samples was determined by immunohistochemistry. All tissue sam-
amples were collected from identical areas of vulva and vestibule in study group and controls. Tissues were embedded in paraffin, cut into sections three- to five-µ thick and incubated with the following antibodies: TNF-α antibody (dilution 1: 400), IL-1β antibody (dilution 1: 200), and IL-1 receptor antibody (dilution 1: 200). The tissue sections were then incubated with anti-mouse antibody followed by exposure to streptavidin, horseradish peroxidase (HRP) conjugate. Finally, 3-amino-9-ethylcarbazole (AEC) was added to serve as a substrate. Afterwards, glass sealed sections were viewed with a fluorescence microscope and photomicrographs were taken using a digital camera. The stained sections were observed by a single investigator who was blinded to the patient data. To avoid false-positive staining, fields were selected at a relative distance from tissue section margins.

Statistical analysis
Statistical analyses were performed using SPSS 15.0 software package. Descriptive and cross-tab analyses were conducted. A value of p < 0.05 was considered statistically significant.

Results
All subjects were Caucasian, from the metropolitan area of Istanbul, low-middle class, and were matched for age.

For TNF-α, positive staining was observed in 40% of the samples from the study group and in 7.7% of the samples from the control group. The difference between the groups was statistically significant (p < 0.05, Figure 1a).

For IL-1β, positive staining was observed in 40% of the samples from the study group, while in 69.2% of the samples from the control group. Although the positive staining ratio was higher in the control group, the difference was not statistically significant (p = 0.12, Figure 1b).

For IL-1 receptor, positive staining was observed in 53.3% of the samples from the study group, while in 84.6% of the samples from the control group. Although the positive staining ratio was higher in the controls, the difference was not statistically significant (p = 0.08, Figure 1c).

Discussion
The cause of VVS remains puzzling. The dominant theory is that VVS is a neuropathic disorder and involves abnormal pain perception due to sensitization of vestibular nerve fibers and the establishment of a sympathetically supported pain loop. Currently unidentified trigger events, probably some type of chronic inflammation, activate and cause extended involvement of the sympathetic, Type C nerve fibers. These fibers are responsible for transmitting noxious chemical or thermal stimuli to the brain. This process leads to an abnormal response of the wide dynamic range neurons in the brain. Hence, mild stimuli are perceived as pain by the patient. Consequently, there is first localized pain of VVS which progresses to the chronic, generalized vulvar pain of dysesthetic vulvodynia [15].

Neuropathic pain happens due to damage or inflammation of the nervous system [16, 17]. Evidence suggests that proinflammatory cytokines, like IL-1β and TNF-α, contribute to the pathogenesis of neuropathic pain [10, 11]. These cytokines are upregulated in the injured peripheral nerves, stimulating chronic neuroinflammation and leading to neuropathic pain [18, 19].

Blocking TNF-α pharmacologically and genetically has prevented neuropathic pain in various studies involving neuropathic pain models [20, 21]. Additionally, some studies presented that administration of TNF-α into the peripheral pain transmission pathways produces a pain response like neuropathic pain [22]. Observed elevated levels of TNF-α in tissue samples of patients with VVS in the present study are in line with the aforementioned association between VVS and neuropathic pain.

IL-1β is a well-characterized cytokine, which is produced by macrophages, and Schwann cells, activating other inflammatory cells [23]. Accumulating evidence suggests that IL-1β may play a vital role in the generation of mechanical hyperalgesia [24]. Considering the well-localized hyperalgesia of the vestibule, the non-significant lower levels of
IL-1β in tissue samples of patients with VVS in comparison to controls in the present study is unanticipated. The low concentration of IL-1β might suggest that cytokines may not be the common pathway to hyperalgesia. Cytokines might increase regionally as a response to trauma and/or inflammation. Of course, the present unanticipated findings could be based upon artefactual differences in cytokine levels at different sampling sites as well.

In studies with animal models, inflammatory hyperalgesia was prevented by administration of endogenous IL-1 receptor antagonist (IL-1ra) [25, 26]. These studies suggested that neutralizing antibodies to IL-1 receptors reduced pain-associated behavior in mice with experimental neuropathy. Interestingly, reduced induction of IL-1ra was observed in the blood samples of patients with VVS compared to controls [27]. Similarly, IL-1 receptor levels were lower in tissue samples of patients with VVS in the present study.

In conclusion, a limitation of the present study was the relatively small sample size. Hence, these findings cannot be generalized to the wider patient group. However, the present authors’ intention was simply to propose that the local inflammation may be mediated by cytokines like TNF-α may rather than trying to single out a pathogenesis of VVS. The findings of elevated TNF-α may suggest new therapeutic alternatives for VVS like inhibiting cytokine synthesis or antagonism of the cytokine receptor.

References

MRI and MRI 3-D reconstruction of anatomic characteristics of the cardinal and uterosacral ligaments in uterine prolapsed women

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Summary

Objective: This study aimed to evaluate the anatomic alterations of the cardinal ligaments (CL) and uterosacral ligaments (USL) in women with uterine prolapse by magnetic resonance imaging (MRI) and MRI three-dimensional reconstruction (3DR). Materials and Methods: Forty patients with uterine prolapse and 40 volunteers with normal support underwent thin layer scan MRI. The 3D models were reconstructed with MRI data and 3D software. Origin, inserted end, geometric shape features of the CL and USL, were compared between the two groups, and the correlation in study group between the MRI and surgical dissection were reported. Results: In the study group, trauma was found in the USL in the insertion or origin. The dorsal USL attached to the sacrum in four (10%) patients of the control group. There was no significant difference in the inserted end of the USL between the two groups, nor in the origin and inserted end of the CL. In the study group, MRI and MRI 3DR better evaluated the anatomic characteristics of the USL compared to intraoperative detection via laparoscopy. Discussion: The approach using MRI and MRI 3DR can non-invasively detect the anatomic abnormality associated with the USL in uterine prolapsed women and can be a useful preoperative planning tool.

Key words: Cardinal ligament; Uterosacral ligament; Pelvic floor dysfunction; Uterine prolapse; MRI three-dimensional reconstruction.

Introduction

Uterine prolapse is a distressing disease which greatly affects women’s daily activities and quality of life [1, 2]. The uterosacral ligaments (USL) and cardinal ligaments (CL) flexibility weakened and anatomic displacement may lead the uterus to descend into the vaginal cavity [3]. It is vital for reconstructive surgery to demonstrate how key anatomic damage of the CL and USL results in uterine prolapse. USL and CL anatomical damage has been studied in cadavers and during surgery, however the syntopy differs from the living state due to the alterations and the borders of the ligament are difficult to establish on surgical dissection [4].

Magnetic resonance imaging (MRI) as main image research technique of pelvic floor dysfunction (PFD), that allows excellent soft tissue resolution and non-invasive depiction and with little distortion. MRI three-dimensional (3D) models can visualize complete shape and continuous changes of the pelvic support structure in living women. However most studies focused on the levator ani muscle (LA) [5-11]. The studies on USL and CL were limited to normal support in women [4, 12-16], hence still insufficient in understanding uterine prolapse.

Therefore, the purpose of this study, was to use thin-layer MRI and MRI 3D technique, to explore main anatomical features of the USL and CL in uterine prolapsed women, in order to supply the objective bases for the surgery related rehabilitation of uterine prolapse.

Materials and Methods

This prospective study was carried at the department of Gynecology and Obstetrics, Southwest Hospital, the Third Military Medical University of Chongqing, China. From October 2010 and December 2011, 40 patients with uterine prolapse in at least Stage 2 were included in the study group, who desired corrective surgery of uterine prolapsed, 40 normal women without symptoms of PFD and previous surgeries in control group. Basic demographic data was collected including age, parity, BMI, menopausal status. All women underwent clinic examination including the Pelvic Organ Prolapse Quantification (POP-Q) [17], Richardson’s examination [18], and urodynamics. Forty patients included 23 cases with anterior vaginal wall prolapsed, 11 cases with paravaginal defects, 22 cases with posterior vaginal wall prolapse, and three cases with stress urinary incontinence (SUI). This study was conducted with approval from the Ethics Committee of Southwest Hospital, the Third Military Medical University. Written informed consent was obtained from all participants.

All the data were obtained by using a 3.0 T MR scanner and a four-channel body phased-array coil. The images were scanned in the transverse and coronal planes at rest. MRI haste technique was used to image the sagittal plane at rest and strain (Valsalva). The transverse and coronal plane images were obtained with sequences of turbo spin echo (TSE) technique T2WI, repetition time [TR] 3100 ms, echo time [TE] 30 -33ms, 0-mm gap, field of view 20×20 cm, matrix 376 × 512, stimulation 2. The transverse plane included images at one-mm slice thickness and the coronal plane...
images at four-mm slice thickness. The MR examination time was approximately 35 minutes.

Transverse images were imported into a 3D imaging program and aligned using bony anatomic landmarks. The gray and scale were adjusted to identify the borders of ligaments [19]. Manual segmentation of MR images of the USL, CL, and pelvic organs on transverse plane was performed. A 3D model of the USL, CL and pelvic organs was also reconstructed. The models’ smooth surface was adjusted in order to avoid distortion.

Results

Baseline characteristics of the two groups population.

In the study, there was no significant differences in age, BMI, pregnant times, labour times, and postmenopausal number between the two groups ($p > 0.05$) (Table 1).

![Figure 1](image1)

Figure 1. — Transverse scan at the level of the cervix and coccyx in healthy women. The images demonstrate origins and insertions of the USL and CL. USL originated from Panel a: piriformis muscle, Panel b: coccygeus muscle; Panel c: ischial spine. CX = cervix, R = rectum, W = whirlbone, IS = ischial spine, C = coccyx, S = sacrum, P = piriformis muscle, CM = coccygeus muscle, CL = cardinal ligament, USL = uterosacral ligament.

![Figure 2](image2)

Figure 2. — Transverse scan in uterine prolapsed women. The images demonstrate defects of the USL on MR. Panel d, at the level of the vaginal and coccyx, the right USL detachment from vaginal vault at the white arrowheads. Panel e, at the level of the cervix, ischial spine and coccyx. The right USL is thinner than the left, the left USL split from ischial spine. Panel f, at the level of the cervix and coccyx. the right USL is thinner than the left. B = bladder, VV = vaginal vault, CX = cervix, R = rectum, W = whirlbone, IS = ischial spine, C = coccyx, CL = cardinal ligament, USL = uterosacral ligament.

<table>
<thead>
<tr>
<th>Head</th>
<th>Number</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Pregnancy (times)</th>
<th>Labour (times)</th>
<th>Postmenopausal (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine prolapse</td>
<td>40</td>
<td>53.1 ± 8.3</td>
<td>23.5 ± 1.1</td>
<td>3.43 ± 1.2</td>
<td>2.23 ± 1.3</td>
<td>28</td>
</tr>
<tr>
<td>Normal support</td>
<td>40</td>
<td>47.8 ± 9.5</td>
<td>22.7 ± 1.4</td>
<td>3.24 ± 1.8</td>
<td>2.74 ± 0.9</td>
<td>26</td>
</tr>
</tbody>
</table>

- **Anatomic characters of the USL and CL based MRI and MRI 3D in uterine prolapse**
- From the MRIs and 3D models viewed, 40 cases were found with anatomical and structural alterations of the USL, with paravaginal defects in ten cases, and levator ani (LA) torn in 30 cases.
- The patients with uterine prolapse MRI are shown in Figure 2 (Panel 1 d-f). The USL was best shown in transverse plane, the CL was easily identified in coronal plane. The USL appeared as thin strips and the CL as vascular sheath. Compared to normal women (Figure 1: Panel a-c), the geometric changes of the USL can occur via a variety shapes (Panel d-f, m). 1) the attachment defects, (e.g.) the USL in the insertion end of cervix or vaginal vault was split, Origin
point detached from the ischial spine or sacrospinous complex was found with trauma and avulsion. 2) bilateral USL were found with an asymmetric origin, in the unilateral continuity was interrupted or thinner. 3) unilateral USL was absent. The CL originated on the pelvic sidewall at the level of the greater sciatic foramen and distributed in cervix, bladder and upper-third of the vagina.

From MRI 3D model of the normal women groups Figure 3 (Panel g-i), the complete shape and geometric characters of the CL and USL can be seen; the origin and

<table>
<thead>
<tr>
<th>Table 2. — Comparison of the origin and insert end of the USL between the uterine prolapsed and normal women</th>
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<tr>
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<tr>
<td>Uterine rolapse</td>
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<tr>
<td>Normal support</td>
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<tr>
<td>$\chi^2$</td>
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<td>$P$ value</td>
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*the origin of the USL; #the insert end of the USL.

<table>
<thead>
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<th>Table 3. — Comparison of the origin and insert end of the CL between the uterine prolapsed and normal women</th>
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<td>Number</td>
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<td>Normal support</td>
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<tr>
<td>$\chi^2$</td>
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<tr>
<td>$P$ value</td>
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</table>

*the origin of the CL; #the insert end of the CL.
Figure 5. — MRI, MRI 3D models and the intraoperative detection of laparoscope demonstrate the relations between the USL, CL, uterus, IS and ATFP in a 43-year-old woman with uterine prolapse. Panel m, MRI demonstrates the left USL detached from cervix; it is difficult to identify the origin. Panel n, MRI demonstrates the left LA avulsion and split. Panel o, MRI demonstrates compared to the right, the left ATFP with a larger grid gap. Panel p, MR3D model shows from left side, the left USL that is absent. Panel q, MR3D model shows from left side, the relations between uterus, CM, ATFP (blue), and LA, and relations between ATFP, cervix, vagina, and urethra. ATFP shape is thin and weak, with attachment to cervix which is not compact, and vaginal side suspension is weak. Panel r, MR3D model as seen from behind. Panel s, MR3D model from left behind demonstrates the relations between uterus, ATFP, and LA. Panel t, the intraoperative detection of laparoscope demonstrates the left USL detached from cervix; it is difficult to assess the origin. The right USL is clearly seen. Panel u, dissection shows the right USL origined from IS. Panel v, the intraoperative detection of the right ATFP detachment from the ischial spine.

CX = cervix, V = vaginal, R = rectum, U = urethra, B = bladder, IS = ischial spine, CM = coccygeus muscle, LA = levator ani, CL = cardinal ligament, USL = uterosacral ligament, ATLA = arcus tendineus levator ani, ATFP = arcus tendineus fasciae pelvis.
inserted end of the ligaments, the anatomical association of ligaments with uterus, rectum, LA, sacrum, ischial spine and ATFP can also be observed. Compared to normal women, (Figure 4, Panel j) MRI 3DR of uterine prolapsed patients, the USL changed arcus shape into “Y” or “V”. The origin and inserted end of the USL and CL are shown in Tables 2 and 3. There was significant difference in the dorsal USL attached to sacrum between the two groups. There was no significant difference in the inserted end of the USL between the two groups, nor in the origin and inserted end of the CL.

**Intraoperative anatomic characters of the USL and CL in uterine prolapse**

Through intraoperative detection with a laparoscope, 22 cases of 40 patients with uterine prolapse were found with anatomical general changes of USL and 13 cases with paravaginal defects. Surgical dissection could not detect the USL avulsion completely, and borders of the ligaments were difficult to establish on dissection, and also difficult to define their origin and insertion.

Figure 5 shows a 43-year-old patient with uterine prolapsed Stage II and anterior vaginal wall prolapse Stage II. In the intraoperative detection with a laparoscope (Panel t), the left USL was visualized with a complete interrupted shape and its origin and border could not be assessed. On dissection, the right USL originated from ischial spine (Panel u); the right ATFP (Panel v) was detached from the ischial spine with avulsion.

**Discussion**

The female normal pelvic floor is a balanced and interrelated system, composed of muscle, connective tissue (CT), ligaments, and nerve components. The cardinal/uterosacral complex comprises level I (apical) suspension, which is critical to pelvic organ support. Any alteration can lead to uterine and the vaginal vault prolapse [1, 3, 4]. The USL and CL have been studied both in cadavers and during surgery. However in cadaveric studies, muscle tension is lost and spasticity is maintained; hence, there is some difference between the cadaver and living body in the pelvic morphology and geometry parameters [20]. In living women, the anatomic borders of the ligaments are difficult to definitely establish on dissection. MRI has been widely used as a quantitative evaluation technique for the pelvic floor. The USL cannot easily be distinguished from pelvic soft tissues by traditional MRI techniques, therefore MRI study on anatomical characters of the USL and CL in living women with uterine prolapse is still limited [4, 12-16].

The speciality of this study is using thin-layer MRI and MRI 3D technique to demonstrate anatomical geometry characters of the USL and CL in women with and without uterine prolapse. Traditional MRI study used pelvic MRI of four- to five-mm slice thickness sequences, in which the borders of pelvic organ and soft tissues were relatively clear, but the deficiency is that the scan layers are fewer, hence the torn ligaments can only be partly observed, and the MRI 3D reconstruction model is somewhat distorted. Research on MRI with thin layer sequence (one-mm slice thickness), can visualize the origin and inserted end of the ligaments, and identify borders especially from rectum fascia. The MRI 3D can accurately demonstrate anatomical characters and displacement of the USL and CL, which is appropriate to observe the anatomical defects and pathological changes of ligaments.

From MRI and MRI 3DR, the preliminary findings regarding the USL in different stage of uterine prolapse include some geometric changes, bilateral asymmetric USL or unilateral USL with avulsion or unilateral absence. The authors also detected its origin or insert end defects, detached or avulsion from the cervix, vaginal or the ischial spine/sacrospinous complex. The patients in which the USL origined from sacrum (4/40) were significantly different from that in normal women (12/40). Meanwhile, the authors detected different stages of paravaginal defect and LA avulsion on MRI and MRI 3DR, consistent with the Petros’ integral theory. They found the intraoperative detection of laparoscope are difficult to dissect the borders of the ligaments, and are difficult to avoid some intraoperative iatrogenic defects.

Above all, the advantages of MRI and MRI 3D technique such as high soft tissue resolution, non-invasive-ness, etc. supposedly to be a preoperative evaluation system of POP, can aid in surgical planning [21], which has a great value for personalized reconstruction of uterine prolapse.

The authors also found the important pole of the ischial spine in pelvic support structure, which is not only attachment point to sacrospinous complex, ATFP, and ATFR, but also origin of a part of the USL. The ischial spine is not only the junction of the pelvic levels I and II, but may also be the mechanical transmission fulcrum. Associated connective tissue around ischial spine is the most vulnerable to damage, such as in USLs or ATFP attachment avulsion with forces delivery or due to anatomical tissue defect [21-25]; the principle needs to be confirmed in further study of pelvic finite element analysis. There was no abnormality found in the anatomical characters of the CL.

There are several limitations. This study presents only the preliminary findings in 40 cases of uterine prolapse and the study number is not sufficient. The anatomical characters regarding the USL for example, bilateral asymmetric USL or unilateral USL absence were also found in two cases with normal support. It testifies that USL defects are not the unique reason for uterine prolapse, which can be accompanied by other tissue defects causing uterine prolapsed [27-28].
References


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e-mail: zhiqingliang@yeah.net
Serum neutrophil gelatinase associated lipocalin and plasma nitric oxide levels in healthy and preeclamptic pregnant

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⁴Istanbul University, Department of Industrial Engineering, Istanbul (Turkey)

Summary

Aims: The authors aimed to evaluate serum neutrophil gelatinase associated lipocalin (NGAL) and plasma nitric oxide (NO) levels in preeclamptic and healthy pregnant women above 24 gestation weeks. Materials and Methods: Forty-nine healthy and 21 preeclamptic (total 70) pregnant women participated voluntarily in the study. Presence of 140 mmHg and above systolic and 90 mmHg and above diastolic blood pressure which emerges after 20th gestation week, proteinuria more than 300 mg/24 hour, and edema were used as diagnostic criterion for preeclamptic pregnant women. Measurements of serum NGAL and plasma NO were performed with enzyme linked immunosorbent assay (ELISA) and photometric method, respectively. Results: Serum NGAL and plasma NO levels of healthy and preeclamptic pregnant groups did not show a statistical difference. In preeclamptic group, a statistically meaningful correlation was found between level of NGAL and body mass index (BMI) of sampling time, creatinine and NGAL, total protein and NO, and albumin and NO. Conclusions: Serum NGAL levels, correlated with serum creatinine levels in this study, may be the early marker of renal damage which may develop mainly due to inflammation and endothelial damage. The authors could not find a statistical difference for serum NGAL and plasma NO levels between healthy pregnant and preeclamptic groups. Varieties peculiar to humans in preeclampsia, impossibility of obtaining first trimester tissue material as an evidence of inadequate trophoblast invasion, and different appearance of maternal reaction to underlying main pathology in every case may restrict clarification of etiopathogenesis.

Key words: Neutrophil gelatinase associated lipocalin; Nitric oxide; Preeclampsia.
Systolic blood pressure of 140 mmHg or above, diastolic blood pressure (mmHg) (100.0-130.0) (140.0-180.0)

### Table 2. — Comparison of laboratory findings in healthy and preeclamptic gravid groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy group (n=49)</th>
<th>Preeclamptic group (n=21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD</td>
<td>mean (min-max)</td>
<td>mean±SD</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>74.0 (61.0-108.0)</td>
<td>97.0 (60.0-285.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>14.0 (8.0-25.0)</td>
<td>24.0 (12.0-42.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.60 (0.30-0.90)</td>
<td>0.80 (0.50-1.40)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T. Chol (mg/dl)</td>
<td>248.71 ± 36.89</td>
<td>291.57 ± 82.24</td>
<td>0.031</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>195.0 (89.0-478.0)</td>
<td>283.0 (124.0-602.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>65.0 (38.0-126.0)</td>
<td>76.29±16.41</td>
<td>0.030</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>136.0±31.08</td>
<td>146.0 (90.0-320.0)</td>
<td>0.082</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>19.4±5.38</td>
<td>27.0 (14.0-346.0)</td>
<td>0.012</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>13.0±4.90</td>
<td>20.0 (5.0-235.0)</td>
<td>0.029</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>73.0 (37.0-156.0)</td>
<td>155.7±79.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>9.0 (4.0-34.0)</td>
<td>12.0 (4.0-139.0)</td>
<td>0.032</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>197.76±31.5</td>
<td>235.0 (131.0-983.0)</td>
<td>0.018</td>
</tr>
<tr>
<td>T. Protein (g/dl)</td>
<td>6.7 (5.0-7.80)</td>
<td>6.02±0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.5 (3.0-4.0)</td>
<td>3.0 (2.10-3.40)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T. Bil. (mg/dl)</td>
<td>0.31 (0.11-0.76)</td>
<td>0.41 (0.17-2.12)</td>
<td>0.11</td>
</tr>
<tr>
<td>D. Bil. (mg/dl)</td>
<td>0.06 (0.02-0.25)</td>
<td>0.05 (0.01-0.53)</td>
<td>0.536</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>137.5±2.24</td>
<td>138.0 (132.0-141.0)</td>
<td>0.751</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>4.0 (3.50-4.70)</td>
<td>4.2 (3.50-5.0)</td>
<td>0.085</td>
</tr>
<tr>
<td>TSH (miU/ml)</td>
<td>2.07±1.03</td>
<td>2.12 (0.08-13.9)</td>
<td>0.604</td>
</tr>
<tr>
<td>NGAL (ng/ml)</td>
<td>124.68 (7.42-218.82)</td>
<td>120.44±50.88</td>
<td>0.078</td>
</tr>
<tr>
<td>NO (µM)</td>
<td>39.83±15.84</td>
<td>37.07±14.48</td>
<td>0.496</td>
</tr>
</tbody>
</table>

### Results

Demographic and clinical findings of healthy and preeclamptic groups are shown in Table 1 and laboratory findings in Table 2.
Table 3. — Correlations between NGAL and NO levels with demographic and clinical findings in healthy and preeclamptic gravid groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy group</th>
<th></th>
<th>Preeclamptic group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGAL</td>
<td>NO</td>
<td>NGAL</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>p</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>Systolic B.P.</td>
<td>0.061</td>
<td>0.675</td>
<td>-0.028</td>
<td>0.850</td>
</tr>
<tr>
<td>Diastolic B.P.</td>
<td>0.071</td>
<td>0.628</td>
<td>-0.106</td>
<td>0.470</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>0.103</td>
<td>0.481</td>
<td>0.436</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI at sampling</td>
<td>0.064</td>
<td>0.663</td>
<td>0.380</td>
<td>0.007</td>
</tr>
<tr>
<td>Infant birth weight</td>
<td>0.231</td>
<td>0.110</td>
<td>0.196</td>
<td>0.178</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.075</td>
<td>0.607</td>
<td>0.148</td>
<td>0.312</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>0.014</td>
<td>0.925</td>
<td>0.225</td>
<td>0.120</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>0.113</td>
<td>0.441</td>
<td>-0.042</td>
<td>0.773</td>
</tr>
<tr>
<td>Gestational age at sampling</td>
<td>-0.171</td>
<td>0.240</td>
<td>-0.127</td>
<td>0.385</td>
</tr>
<tr>
<td>Protein in spot urine (mg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

B.P.: Blood pressure

Table 4. — Correlations that was founded meaningful between NGAL and NO levels with other laboratory findings in healthy and preeclamptic gravid groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy group</th>
<th></th>
<th>Preeclamptic group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGAL</td>
<td>NO</td>
<td>NGAL</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>p</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.09</td>
<td>0.537</td>
<td>0.055</td>
<td>0.706</td>
</tr>
<tr>
<td>T. protein (g/dl)</td>
<td>-0.198</td>
<td>0.173</td>
<td>0.165</td>
<td>0.258</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>-0.146</td>
<td>0.318</td>
<td>-0.064</td>
<td>0.66</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>-0.418</td>
<td>0.003</td>
<td>-0.037</td>
<td>0.803</td>
</tr>
</tbody>
</table>

Correlations between NGAL and NO levels with demographic and clinical findings in healthy and preeclamptic gravid groups are shown in Table 3.

Correlation coefficients that were founded meaningful between NGAL and NO levels with other laboratory findings in healthy and preeclamptic gravid groups are shown in Table 4. A statistically significant correlation between Na and NGAL variants were detected in healthy group. A statistically significant correlation between creatinine and NGAL; T. protein and NO; albumin and NO were detected in preeclamptic group.

Discussion

Although preeclampsia is one of the most serious complications of pregnancy affecting 8% of whole pregnancies, only a few is known about its etiology [1].

In a study in which NGAL and pregnancy associated plasma protein A (PAPP-A), which are assumed as determinants of endothelial and placental damage, were evaluated in trimester preeclampsia and serum median NGAL levels and were detected higher in preeclamptic group compared to control group and first trimester NGAL sensitivity was found lower than second trimester NGAL sensitivity in estimating late onset preeclampsia. Additionally in this study NGAL levels were found positively correlated with systolic and diastolic blood pressures and proteinuria [2].

Elneihoum et al. [11] in a study, detecting serum NGAL levels were correlated with increased blood pressure; this result was reported as consistent with a previous study in which NGAL levels were detected higher in non-pregnant hypertensive women in comparison to healthy controls and correlated positively with diastolic blood pressure.

In the study of D’Anna et al. [12], serum NGAL levels were detected higher in second trimester of preeclamptic gravids than that of normal gravids and a positive and high correlation between blood pressure and proteinuria with NGAL was observed in preeclamptic group.

Arikan et al. [13], detected rather lower plasma lipocalin-2 levels in preeclamptic gravids than healthy gravid group and they reported that this findings can display the role of lipocalin-2 in pathogenesis of preeclampsia.

D’Anna et al. [2], serum median NGAL levels were detected higher in preeclampsia than control group and in predicting late onset preeclampsia, although the sensitivity of first trimester NGAL was detected lower than that of second trimester NGAL, it was reported that first trimester serum NGAL was an early indicator of late onset preeclampsia.

In the present study there was no difference between healthy and preeclamptic gravids in terms of serum NGAL levels.

D’Anna et al. [12] detected a significant and positive correlation between NGAL and proteinuria in preeclamptic gravids. However, while the present authors could not find a correlation between serum NGAL and protein levels in spot urine in preeclamptic group, they detected a significant
positive correlation between serum creatinine and serum NGAL levels. On the other hand, serum creatinine levels were detected significantly higher in preeclamptic group than healthy group. This finding is consistent with that of Nickolas et al. [7] who claimed serum NGAL levels were early indicators of renal damage, therefore serum NGAL levels correlated with serum creatinine in preeclamptic gravids could be early indicators of renal damage.

Seligman et al. [14] performed a study in 26 preeclamptic and 26 normotensive gravids to search the role of NO in preeclampsia. They detected significantly lower serum nitrite and nitrate levels in preeclamptic gravids than control group. Similarly, Li et al. [15] studied cGMP levels that is the second messenger for NO. When compared to control group, nitrite, nitrate and cGMP levels in preeclamptic gravids were significantly lower.

Mao D et al. [10] reported they detected significantly lower plasma NO levels in preeclamptic gravids in comparison to healthy gravids. However in some studies concerning preeclamptic gravids, conflicting results which were higher, lower and unchanged were obtained [14, 16, 17].

As a result the present authors did not observe significant differences between preeclamptic and healthy gravids terms of NGAL and NO levels. The studies concerning this issue have been conflicting results. The studies performed with larger preeclamptic gravid sampling groups are needed.

References


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Investigation and analysis of contraceptive measures towards different reproductive-aged women in Yangzhou

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Summary

Objective: This study aims to retrospectively investigate the contraceptive methods of different reproductive-aged women in two different regions of Yangzhou, China, to understand the changes of the contraceptive methods, and to analyze the targeted improvements of future informed choices of contraception and birth control, and reduce the occurrence of the unintended pregnancies. Materials and Methods: This study included 13,407 and 20,876 married reproductive-aged women (MCW) that were chosen from a city downtown and a county-level city, respectively, for the group-organized random sampling questionnaire survey. Results: There was a significant urban-rural difference in the choice of contraceptive methods in different MCW. The female choices of contraception were single; the accessibility of contraceptive supplies, and personalized services could improve the implementation rate of long-term contraceptive measures. The cooperation of male contraceptive responsibility was related to the education levels and enhanced male-female communication. Conclusions: It was very important for urban and rural women with different ages to choose different contraceptive measures, suggesting that these choices require the development of different educational models towards women of different ages and regions. The accessibility to various aspects of counseling and contraceptive measures should be strengthened, meanwhile male participation should also be promoted to prevent unwanted pregnancies and to reduce the health hazards related to sexual activities.

Key words: Reproductive-aged women; Regional age distribution; Contraceptive measure.

Introduction

From the perspective of maintaining, protecting, and developing civil rights, the concept of offering informed choices regarding contraception has generally been carried out more than ten years. The Nation supplies the reproductive-aged people with free contraceptives drugs and devices from four categories and 31 varieties and specifications [1]. The relative service offered has formed a high-quality procedure in providing contraceptive measures, while helping to raise the awareness of contraceptive knowledge among reproductive-aged women, while helping them to make independent, voluntary, and responsible decisions based on the full understanding of the situation. However, with the gradual development of the informed choice, the short-acting self-controlled contraceptive measures have been more and more preferred among the contraceptive choices of married reproductive-aged people [2]. The termination option rate and the replacement rate of female contraception increase, while the male participation rate is still low, and as the sexual demands of adolescent increase, the phenomenon of noncontraception also increases, resulting in the incremented unplanned pregnancy rate and related consequences. Contraception is an important measure to reduce unwanted pregnancies [3]. Many studies have demonstrated that the cultural background and ethnic role are the determinative factors of childbirth and contraceptive behavior [4, 5]. Therefore, it would be necessary to understand the contraceptive-method status of women under the cultural background of Yangzhou region, Jiangsu Province, to compare the variation rules of contraceptive measures according to different ages, and to analyze the targeted measures towards the informed choice of contraceptive methods, while helping women to choose safe, effective, and appropriate contraceptive measures, and to reduce unwanted pregnancies.

Materials and Methods

Subject

During the implementation procedure of Jiangsu Provincial cervical Cancer Screening Project, two counties (city, district) of Yangzhou were set as the project sites. The married reproductive-aged women in the project sites that were investigated were 34,292 and 34,283 questionnaires were valid, among which Guanling district, Yangzhou downtown, had 13,407 questionnaires, with an average age of 45.6 years; Gaoyou, Yangzhou county, had 20,876 questionnaires, with a mean age of 45.3 years. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Hospital of Maternal and Child Health. Written informed consent was also obtained from all participants.

Survey methodology

The project screening fee was paid by the government. The married reproductive-aged women, living within the target region, were chosen. The group-organized random sampling was performed for the investigations. The investigators were trained professional technical persons responsible for the effective questionnaire-filling. The survey was conducted from January 2009 to
December 2009. The patients were divided into two types: contraceptive measures currently used (taking relatively long-lasting and high efficient contraceptive measures during survey) and contraceptive measures used in the past (had used other contraceptive measures before survey). In addition, the constitute situation of the contraceptive methods used by the reproductive-aged women in the above regions in 2009 were statistically analyzed. The regional relative female population data were obtained from the 6th census data according to the Bureau of Statistics in 2010.

Statistical analysis
EpiDate software was used to build the database, and the data were entered into for summary. SPSS15.0 software was used for the statistical analysis, with the chi-square (χ²) test towards the categorical variables. Statistical significance was considered when \( p < 0.05 \).

Results
General demographic characteristics
The basic survey was performed towards the ratio of the population of the two study groups in the regional female population, and the results showed that there were less people in the 20 to 29 age group, which was related with the fact that the unmarried persons did not participate in the survey (Table 1).

There were no statistical differences in the points of age composition (\( \chi^2 = 2.756 \)) and marital status (\( \chi^2 = 1.895 \)) in the age subgroup of 20–65 years (\( p > 0.05 \)). As for the educational levels (illiteracy, primary school, secondary school, college, and above), the data in the city downtown were 9.64%, 16.62%, 62.83%, and 10.91%, respectively, and the data in the rural region were 39.30%, 24.19%, 35.48%, and 1.03%, respectively, with statistical significance (\( \chi^2 = 5.765, p < 0.01 \)).

Contraceptive measures currently used
The women under 40 years in the two regions concentrated on the use of intrauterine device (IUD) and condom, accounted for nearly 90%; the ratios of women under 30 years who used IUD in downtown were 35.7% and 71.51% in the county; the ratio of condom-usage in downtown was 54.09%, and 19.59% in the county. In the 20~49-year group, the ratio of the use of condoms decreased 50% in downtown, and 20% in the county; when over 50 years of age, the ratios of non-using contraceptive measures were 75.33% in downtown and 11.32% in county (\( \chi^2 = 3.804, p > 0.05 \)). In the county, the ratio of male and female sterilization reached 84.71%; when over 50 years of age; the ratios of IUD were 13.11% in downtown and 3.67% in the county (Table 2).

Contraceptive measures used in the past
The ratios of before 30-year-old females who used one contraceptive method (i.e., never-used was subtracted by the currently unused) were 61% and 54.8%, respectively (\( \chi^2 = 0.875, p > 0.05 \)); before 40-years, the ratios were 48.8% and 31.4% (\( \chi^2 = 3.804, p > 0.05 \)); before 50-years, the ratios were 23.3% and 21.1%, respectively, (\( \chi^2 = 1.2515, p > 0.05 \)), without significant difference (Table 3).

Current situation
Among the entire married reproductive-aged women (15–49 years of age) in the city, the ratio of taking contraceptive measures in county accounted for 90.82%, and 87.64% in downtown; among the current measures, the ratio of using long-term measures accounted for 80.25% in county, and 67.38% in downtown; the current usage of the
Investigation and analysis of contraceptive measures towards different reproductive-aged women in Yangzhou

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contraceptive drugs in both groups was less than 1%; the current condom usage rates were 19.89% in downtown and 9.44% in the county (Table 4).

Discussion

In this study, there was no significant difference in age or marital status between the two study groups, except in the level of education. As consistent with other city, the implementation rate of long-term contraceptive measures in Yangzhou exhibits a downward trend. The implementation rate of sterilization decreased year by year, and that of IUD remains stable [6]. The attitude of women in Yangzhou to various contraceptive methods can be learned from their ages.

Using IUD is a safe, highly effective, long-lasting means of contraception [7]. Internationally, IUDs are the most widely used reversible method of contraception [8]. On an average, 15% of reproductive-aged women in developing countries and 8% in developed countries use IUDs [8]. Chinese IUD is the basic contraceptive item, with the policy of free supplication and high popularity, and the current using rate of IUD in Yangzhou also reaches 70%. The usage rates of IUD in young-age groups were 71.51% and 75.03% in county, higher than those in downtown (35.69% and 63.05%), the difference gap was 12% to 35%, which trended to be equal (60%) in 40-year-old group and above. The usage rate in over 50-year-old population in Yangzhou is 13.11%, greatly higher than the 3.67% in county. This indicates that there is difference of popularization of contraceptive method informed choice between city and rural region. The self-selection sense of women in downtown was strong, while the actual implementation was poor. They seldom turned to professional service workers for help. The women in county often communicated mutually, and the service workers often went to their families to implement the contraception. Therefore, the necessary measures should be taken in downtown women, to improve the awareness and implementation rates of contraception.

The use rate of condoms reflects the trend of a younger population. In the survey, the population of using contraceptive film, subcutaneous implantation, and contraceptive injection was small, while the short-acting contraceptive measures were mainly condoms; the rate in 20– years group reached 54%, which had important relations with independent choice and tendentious guide of obstetrics and gynecology doctors. Almost one-third did not recommend them to unmarried women [9], though the doctors believed that IUD was convenient and effective. Condom use was the only contraceptive measure whose rate was higher than that in the county: 19.89% and 9.44%, respectively. The results of sub-age groups of condom users, both current and former users all showed that before 40 years and after 50 years of age, the rates in the city were higher than that in the county, while in the 40-year group, the rates were similar, indicating that the cooperation of men’s responsibility in contraception was related to the educational level and the enhancement of communication between men and women; the educational level in the city was higher than that in the county, while in the 40-year group, the rates were similar, indicating that the cooperation of men’s responsibility in contraception was related to the educational level and the enhancement of communication between men and women; the educational level in the city was higher than that in the county. Thus, the reduction and contempt of education in men’s contraception should be avoided, which might be conducive to the promotion of gender equity and justice, and to build healthy sexual relations in families and communities.

The low use rates of contraceptive drugs reflected that the current acceptance of contraceptive drugs by population was relatively poor. The current use rates of contra-

<table>
<thead>
<tr>
<th>Table 3. — Contraceptive measures previously used at different ages (%).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
</tr>
<tr>
<td>Downtown</td>
</tr>
<tr>
<td>30~</td>
</tr>
<tr>
<td>40~</td>
</tr>
<tr>
<td>50~65</td>
</tr>
<tr>
<td>County</td>
</tr>
<tr>
<td>30~</td>
</tr>
<tr>
<td>40~</td>
</tr>
<tr>
<td>50~65</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p < 0.01 compared with the county.

<table>
<thead>
<tr>
<th>Table 4. — Current contraceptive methods in the entire city in 2009.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
</tr>
<tr>
<td>Downtown</td>
</tr>
<tr>
<td>(%)</td>
</tr>
<tr>
<td>County</td>
</tr>
<tr>
<td>(%)</td>
</tr>
</tbody>
</table>
ceptive drugs were less than one percent. Since the introduction of the first combined hormonal contraceptive in 1960, there have been many developments toward the goal of minimizing side effects and improving compliance without compromising efficacy [10]. Recent studies have found that the prevalence of unreliable contraception remains steady, and hormonal contraception, despite its availability, has not been widely adopted by women [11, 12]. The knowledge and belief of clinical service providers towards certain methods, and the confidence on the contraceptive methods and risk would affect the current rate and promotion in population. Only a small minority of obstetrician-gynecologist physicians objected to one or more common contraceptive methods or would refuse to offer a contraceptive method requested by a patient [13]. Therefore, efforts to improve the accuracy of information in the media and expand patient-to-patient communication about satisfaction with long-acting reversible contraceptives (LARCs) may improve positive awareness [14]. Healthcare providers are encouraged to counsel patients regarding available contraceptive options and their associated benefits and risks [15]. Females should be aware that oral contraceptives are the initiative methods, reducing unwanted pregnancies because of no contraceptive measures or frustration caused by men’s reluctant to use condoms, protecting reproductive health and increasing quality of life.

The contraceptive choice tends to be simple and reversible. This survey showed that there was an obvious increasing of non-contraceptive measures after the age of 40; the ratios of only using one contraceptive method in the city and county were 55%~61%, 31%~49%, and 21%~23% towards 20-, 30- and 40- age groups, respectively; in 20-49-year-old group, the condom-usage rates decreased with the increasing ages, in city; the rate reached 50%, and the rate was 20% in county, showing that the choices of contraceptive needs towards different reproductive-aged women in each age group were simple, long-lasting and continuative. Although young women trust the information they receive from healthcare providers, the majority do not learn about contraception from this source [16]. Peer-to-peer information transfer is clearly important [17-19]. Therefore, except for one-to-one service, the peer education activities were also suitable for women of all ages to improve the awareness of contraception.

This survey was limited to married people. It could not reflect the elevated unintended pregnancy rate in unmarried young women who did not use or persist in contraceptive methods. EC requestors (compared with non-requestors) were more likely to have engaged in unprotected sexual intercourse is consistent with findings that were reported from family planning clinics [20, 21] and urban high school-based clinics (22). Therefore, clinicians should consider intrauterine contraception in appropriate candidates, including women who are nulliparous, adolescent, immediately postpartum or postabortal, and desiring emergency contraception, and as an alternative to permanent sterilization [23], prompting young people to use contraceptive measures or more readily to use long-acting contraceptive methods.

With the social development, the demand of women’s participation in social activities, in conjunction with increased life events with men, also incremented. However, the cultural tradition of male dominance is still deep-rooted in the society; the main burden and responsibility of contraception is still the women’s responsibility [24]. The appropriate methods of contraception would be convenient while without adverse consequences to women, and are important in protecting the reproductive health of women. All the single contraceptive methods, no continuance in contraception, incidental psychology to no contraception, and tendentious guide of service workers can affect the contraceptive effect. Therefore, it is necessary to improve the communication skills of service workers in medical institutions. The multiple choices of contraceptive methods in adolescent or low-age nulliparous women, and the long-term contraception in women after childbirth or abortion should be strengthened. For women above 40 years of age, contraception should not be easily discontinued. Women of about 50 years of age and menopausal should be guided to timely terminate contraception. The age and educational level should be combined to provide the personalized contraception guidance and convenient contraceptive methods, for improving their accessibility while incrementing unintended pregnancies prevention, and reducing the security of sexual life.

Acknowledgements

The survey data collection was strongly supported and participated by Population and Family Planning Committee and the County Statistical Bureau of Yangzhou, Guangling and Gaoyou. A special appreciation is extended to the above units.

References

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Does the estradiol level on the day of human chorionic gonadotropin administration predict the clinical outcome of controlled ovarian hyperstimulation?

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

Summary

**Objective:** To investigate the effect of serum estradiol (E2) levels on the day of human chorionic gonadotropin (hCG) administration on the outcome of controlled ovarian hyperstimulation (COH) in both long gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist protocols. **Materials and Methods:** This study included 212 in vitro fertilization-embryo transfer (IVF-ET) cycles performed with either long GnRH agonist or GnRH antagonist protocols were classified into three groups according to serum E2 levels measured on the day of hCG injection: < 2,000 pg/ml, 2,000–4,000 pg/ml, and > 4,000 pg/ml. The three groups were compared according to age, number of retrieved oocytes, number of transferred embryos, and pregnancy rates for each of the stimulation protocols. **Results:** The long and antagonist protocols were performed in 130 and 82 cycles, respectively. The pregnancy rates were 21.5% (28/130) and 23.2% (19/82) in the long- and antagonist-protocol groups, respectively. Serum E2 levels were measured on the day of hCG administration as < 2,000 pg/ml in 65 cycles, 2,000–4,000 pg/ml in 76 cycles, and > 4,000 pg/ml in 71 cycles. The number of retrieved oocytes increased in parallel to serum E2 levels (p = 0.001). However, there was no significant difference among groups in the pregnancy rates (p = 0.116). Similarly, the number of retrieved oocytes increased in parallel to serum E2 levels in both of the protocol groups (p value was 0.001 in both long GnRH agonist and antagonist protocols), but there was no correlation between the pregnancy rates and serum E2 levels (p value of long GnRH agonist protocol was 0.254 and the p value of antagonist group was 0.349). **Conclusion:** The serum E2 level on the day of hCG administration does not predict the pregnancy outcome in IVF with either long GnRH agonist or GnRH antagonist protocols.

**Key words:** Infertility; Assisted reproduction; Estradiol; In vitro fertilization; Pregnancy.

Introduction

Endometrial receptivity and embryo quality are two major factors in the implantation process. Estrogen is essential for the preparation of the endometrium for implantation, as it affects both endometrial proliferation and augmentation of uterine and endometrial perfusion. Because supraphysiologic levels of serum estradiol (E2) are reached during controlled ovarian hyperstimulation (COH), the impact of E2 levels on pregnancy and implantation rates has been a topic of concern.

The results of previous investigations have been controversial. Although some studies have suggested a positive correlation between serum E2 levels and pregnancy rates [1, 2], the majority have failed to find any such relationship [3-5]. On the other hand, a decrease in the pregnancy rate due to the detrimental effects of high E2 levels on both uterine receptivity and oocyte/embryo quality has been demonstrated [6, 7].

In most studies on this topic, gonadotropin-releasing hormone (GnRH) agonists were used for the prevention of a premature luteinizing hormone (LH) surge during gonadotropin stimulation [8-10], and GnRH antagonists were utilized in a few studies [11]. In this study, we aimed to investigate the relationship between serum E2 levels on the day of human chorionic gonadotropin (hCG) administration and the outcome of COH in both long GnRH agonist and GnRH antagonist protocols.

Materials and Methods

We analyzed the data of 212 assisted reproductive technology (ART) cycles performed at the Reproductive Endocrinology Unit of the Istanbul Faculty of Medicine from December 2005 to December 2008. They excluded patients younger than 25 and older than 35 years, as well as the cycles in which an embryo transfer was not performed for any reason. Either a long GnRH agonist or GnRH antagonist protocol was preferred for COH based on the patient’s age, ovarian reserve, and previous response to ovarian stimulation. On day 3 of the cycle, serum follicle-stimulating hormone (FSH) and E2 levels were measured.

In the long protocol with a GnRH agonist, one mg of leuprolide acetate was administered on day 21 of the prior cycle. After a baseline ultrasonographic evaluation of the endometrium and ovaries on day 3 of the cycle, either recombinant FSH or human menopausal gonadotropin (hMG) was administered in appropriate dosages for ovarian stimulation, and the GnRH agonist dosage was reduced to 0.5 mg daily. In the GnRH antagonist protocol, ovarian stimulation was begun on day 3 of the cycle, and 0.25 mg of either cetorelix or ganirelix was applied when the leading follicle reached 13 to 14 mm in diameter. Ovarian follicular growth was monitored daily by transvaginal ultra-
sound. hCG was administered when there were at least two follicles >18 mm in diameter. The serum E2 level was measured on the same day as the injection. Oocyte retrieval was performed 36 hours after hCG administration by ultrasound-guided follicular aspiration. Three days after oocyte retrieval, grade 1 and 2 embryos were replaced. Pregnancy was diagnosed by serum concentrations of β-hCG on day 12 of the embryo transfer. Also, transvaginal ultrasound was performed during sixth week of gestation.

Patient characteristics and outcomes of long GnRH agonist- and GnRH antagonist-protocol cycles were compared. The patients were classified into three groups according to serum E2 levels measured on the day of hCG injection: < 2,000 pg/ml (Group 1), 2,000–4,000 pg/ml (Group 2), and > 4,000 pg/ml (Group 3). The three groups were compared according to age, number of retrieved oocytes, number of transferred embryos, and pregnancy rates for each of the stimulation protocols.

Data were analyzed with NCSS 2007 and PASS 2008 statistical software programs. The differences between long and antagonist protocols were assessed using Chi-square for categorized variables, and t-test and Mann Whitney U test for continuous variables. Comparisons according to serum E2 levels on the day of hCG administration were assessed using Chi-square for categorized variables, and one-way ANOVA and Kruskal–Wallis test for continuos variables. The level of statistical significance was defined as p < 0.05, with a confidence level of 95%.

### Results

Demographic characteristics of the patient population are detailed in Table 1. The long GnRH agonist and GnRH antagonist protocols were performed in 130 and 82 cycles, respectively. The overall clinical pregnancy rate per transfer was 22.2%. The duration of infertility was significantly lower (p = 0.039), and serum E2 level and the number of retrieved oocytes were significantly higher in the long GnRH agonist protocol group in comparison to the GnRH antagonist protocol group (p = 0.001 and 0.039, respectively). However, there was no statistically significant difference between the pregnancy rates (21.5% vs. 23.2%, p = 0.781).

Serum E2 levels were measured on the day of hCG administration as < 2,000 pg/ml in 65 cycles, 2,000–4,000 pg/ml in 76 cycles, and > 4,000 pg/ml in 71 cycles (Table 2). The number of retrieved oocytes increased in parallel to serum E2 levels (p = 0.001). However, we found no statistically significant difference among groups in the pregnancy rates (p = 0.116).

Table 3 shows the ART outcomes of the long GnRH agonist-protocol cycles. Serum E2 levels were measured on the day of hCG injection as < 2,000 pg/ml in 30 cycles,
the groups were no significant difference in the pregnancy rates among E2 levels [8, 15]. Suboptimal environment for implantation embryos did not appear to be negatively affected by serum reports have demonstrated that the quality of oocytes and ever, the second mechanism is controversial because some negatively influence the implantation process [14]. How-

derived oocyte number. However, after the optimal concentration is reached, the increasing quantity of retrieved oocytes does not further improve the implantation rates. When a level higher than the optimal level is reached, the detrimental effect on endometrial receptivity seems to reduce pregnancy rates.

Altho

gondotropin, and day-3 FSH and E2 concentrations on pregnancy rates. This supports the theory that high E2 levels do not have a detrimental effect on embryo development, but after an optimal threshold, higher numbers of oocytes do not improve the embryo implantation rate.

In conclusion, the present study showed that serum E2 levels on the day of hCG administration do not predict the pregnancy outcome in ART cycles with either long GnRH agonist or GnRH antagonist protocols, although there is a positive correlation between the hormone level and the number of retrieved oocytes.

References


Table 4. — Comparison of variables between the groups according to serum E2 levels on the day of hCG administration in GnRH antagonist-protocol cycles.

<table>
<thead>
<tr>
<th>E2 levels (pg/ml)</th>
<th>≤2,000</th>
<th>2,000–4,000</th>
<th>&gt;4,000</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 35)</td>
<td>(n = 26)</td>
<td>(n = 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.91 ± 3.10</td>
<td>30.92 ± 2.67</td>
<td>29.67 ± 2.97</td>
<td>0.276</td>
</tr>
<tr>
<td>Retrieved oocyte number</td>
<td>9.20 ± 4.57</td>
<td>14.42 ± 7.56</td>
<td>19.14 ± 5.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Embryo/transfer</td>
<td>2.71 ± 0.62</td>
<td>2.96 ± 0.19</td>
<td>2.90 ± 0.43</td>
<td>0.082</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>22.9</td>
<td>15.4</td>
<td>33.3</td>
<td>0.349</td>
</tr>
</tbody>
</table>

Note: Values are means ± SD

2,000–4,000 pg/ml in 50 cycles, and > 4,000 pg/ml in 50 cycles. The number of retrieved oocytes increased in parallel to serum E2 levels (p = 0.001). Additionally, there were no significant difference in the pregnancy rates among the groups (p = 0.254).

Table 4 shows ART outcomes of GnRH antagonist-protocol cycles. Serum E2 levels were measured on the day of hCG administration as <200 pg/ml in 35 cycles, 2,000–4,000 pg/ml in 26 cycles, and > 4,000 pg/ml in 21 cycles. The number of retrieved oocytes increased in parallel to serum E2 levels (p = 0.001). However, there were no significant differences in the pregnancy rates among the three groups (p = 0.349).

Discussion

It is known that the success of implantation depends on both endometrial receptivity and embryo quality. The hormone levels in natural cycles are ideal for the development of the embryo and endometrium. However, serum E2 levels are at least ten times higher in ART cycles [12]. Recent investigations have suggested that high E2 levels have a detrimental influence on pregnancy outcomes by two different mechanisms. One involves cellular changes in oocytes and the endometrium as a consequence of the alteration of the estradiol–progesterone balance, which decrease the receptivity of the endometrium [13]. The other mechanism is the direct toxic effect of high E2 levels on embryos, which may negatively influence the implantation process [14]. However, the second mechanism is controversial because some reports have demonstrated that the quality of oocytes and embryos did not appear to be negatively affected by serum E2 levels [8, 15]. Suboptimal environment for implantation may be due to the gland–stromal dyssynchrony, which shows a deficient secretory endometrial transformation of the endometrium [16]. The color Doppler analysis of peri-implantation endometrial perfusion showed that high estradiol concentrations after ovarian stimulation impaired uterine blood flow and implantation [17, 18]. Ma et al. stated that the higher levels of estrogen cause the window of uterine receptivity to close rapidly, in contrast to the lower levels of estrogen in which the window of receptivity re-

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Relation of red cell distribution width to the presence and severity of endometriosis

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Summary
Aim: Although the exact pathogenesis of endometriosis is not known, it is proposed to be a chronic inflammatory disease. The association between red cell distribution width (RDW) and inflammation is well established. Therefore, in the present study, the authors aimed to investigate the association between presence and severity of endometriosis and RDW. Materials and Methods: Fifty endometriosis patients and 48 controls were included in the study. The endometriosis group was categorized in two subgroups as mild-to-moderate (n=35) and moderate-to-severe disease (n=15). CA-125 and RDW values of all participants were measured. Results: Both RDW (17.7 ± 2.2 vs 14.9 ± 1.5, p < 0.001) and CA-125 (50.6 ± 35.1 vs 27.9 ± 4.8) levels were significantly higher in the endometriosis patients when compared to the control group. Moreover, the authors found a significant positive correlation between RDW and CA-125 levels (r: 0.495, p < 0.001). Conclusion: The present study results demonstrated that RDW levels were significantly increased in endometriosis patients and associated with the severity of endometriosis.

Key words: Red cell distribution width; Endometriosis.

Introduction
Endometriosis is classically defined as the extraterine proliferation of endometrial tissue. It is a common benign gynecologic disease seen in 10% of women at reproductive age which is also observed in 35-50% of infertility patients [1, 2]. Despite the advances in imaging and biomarker technologies, endometriosis is still diagnosed via surgical exploration and histological examination [3]. Although there is no reliable non-invasive test, CA-125 is the most commonly preferred marker [4]. CA-125 is elevated in advanced endometriosis; however, it has a low sensitivity for minimal and mild endometriosis [5]. Although the exact etiopathogenesis of endometriosis is not known, it is regarded as a chronic inflammatory disease [6]. Studies have shown elevated inflammatory markers in the serum and peritoneal fluid of endometriosis patients [7].

Red cell distribution width (RDW) is a hematologic parameter indicating variations in erythrocyte volume. Recently, it has been shown to have a prognostic value in acute and chronic cardiac cases and healthy individuals [8-10]. There appears to be a relationship between chronic inflammation and elevated RDW, although the underlying association is not fully known [11]. In the present study, the authors aimed to investigate the association between endometriosis and RDW.

Materials and Methods
The study population consisted of 98 patients who underwent surgical exploration for primary infertility between February 2010 and December 2012. Out of these, 50 patients were diagnosed with endometriosis by histopathological examination and 48 healthy individuals without endometriosis or other pathology in the laparoscopy were enrolled as the control group. The patients with a history of an operation within the past six months as well as those with hormonal therapy, anemia, or systemic disease (eg, diabetes or hypertension), inflammatory diseases, kidney disorders, and signs of other concurrent medical complications were excluded from the study. The endometriosis group was classified according to the American Society of Reproductive Medicine as follows: minimal-to-mild disease (Stage 1-2; n=35) and moderate-to-severe disease (Stage 3-4; n=15) [12]. This study was approved by the local ethics committee and informed consents were obtained from all participants. All participants underwent blood collection before surgical exploration via antecubital vein puncture. Hemoglobin (Hb), RDW and white blood cell count, and other hematological indices were measured as part of the automated complete blood count (CBC) using a LH 780 hematology analyzer. CA-125 was measured by using electrochemiluminescence immunoassay (ECLIA) and cut off values of CA125 were determined as 35 U/ml.

Statistics
Continuous variables are expressed as mean ±SD. Categorical variables are expressed as percentages. To compare parametric continuous variables, the Student’s t test was used; to compare nonparametric continuous variables, the Mann-Whitney U was used. To compare categorical variables, the chi-square test was used. To assess differences in RDW and CA125 in between the control group, mild to moderate and moderate to severe endometriosis groups, analysis of variance (ANOVA) and Tukey’s Honestly Significant Difference (HSD) as a post hoc test were used. Two-tailed p values < 0.05 were considered to indicate statistical significance. Correlation analyses between variables were performed using Pearson or Spearman correlation. Statistical analyses were performed using SPSS, version 15.0 for Windows.
Results

The mean age of the study population was 32 ± 3.5 years (33 ± 3.7 in the endometriosis group vs 32 ± 3.3 in the control group; \( p = 0.386 \)). The general characteristics of the study population are shown in Table 1.

In the endometriosis group, RDW ranged from 14.0% to 22.5% (mean 17.7 ± 2.2%), whereas in the control group it ranged from 12.0% to 17.8% (mean 14.9 ± 1.5%, \( p < 0.001 \)). Similarly, CA-125 levels were significantly higher in the endometriosis group than in the control group (50.6 ± 35.1 vs 27.9 ± 4.8; \( p = 0.004 \)). Regarding the endometriosis subgroup analysis, CA-125 level was significantly higher in the moderate-to-severe subgroup than in the mild-to-moderate subgroup (Figure 1, Table 2). However, no significant difference relative to CA-125 level was observed between the control and mild-to-moderate endometriosis groups (27.9 ± 4.8 vs 28 ± 5.5, \( p = 0.930 \)). In addition, the subgroup analysis revealed that RDW level was significantly higher in the moderate-to-severe endometriosis subgroup as compared to the mild-to-moderate endometriosis subgroup (19 ± 2 vs 17.2 ± 2.1, \( p = 0.007 \)) and the control group (19 ± 2 vs 14.9 ± 1.5, \( p < 0.001 \)) (Figure 2). Moreover, a significant difference regarding to the RDW level was also observed in between the mild to moderate endometriosis subgroup and the control group (17.2 ± 2.1 vs 14.9 ± 1.5, \( p < 0.001 \)). Moreover, the authors found a significant positive correlation between RDW and CA-125 levels (\( r = 0.495, p < 0.001 \)) (Figure 3).

Table 1. — Main clinical and laboratory characteristics of the patient and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Endometriosis (n:50)</th>
<th>Control group (n:48)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33±3.7</td>
<td>32±3.3</td>
<td>0.386</td>
</tr>
<tr>
<td>Infertility duration</td>
<td>3.8±0.7</td>
<td>3.7±0.8</td>
<td>0.606</td>
</tr>
<tr>
<td>RDW,%</td>
<td>17.7±2.2</td>
<td>14.9±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ca-125</td>
<td>50.6±35.1</td>
<td>27.9±4.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.2±1.5</td>
<td>11.6±1.6</td>
<td>0.176</td>
</tr>
<tr>
<td>Platelet (x10^3 µl)</td>
<td>269±86</td>
<td>264±78</td>
<td>0.907</td>
</tr>
<tr>
<td>WBC (x10^3 µl)</td>
<td>8.5±1.9</td>
<td>8.2±1.9</td>
<td>0.377</td>
</tr>
</tbody>
</table>

RDW: red cell distribution width; WBC: white blood cell.

Table 2. — Main clinical and laboratory characteristics of the endometriosis subgroups and controls.

<table>
<thead>
<tr>
<th></th>
<th>Stage 1-2 (n:35)</th>
<th>Stage 3-4 (n:15)</th>
<th>Control (n:48)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33±4</td>
<td>32±2.9</td>
<td>32±3.3</td>
<td>0.514</td>
</tr>
<tr>
<td>Infertility duration</td>
<td>3.7±0.8</td>
<td>3.9±0.4</td>
<td>3.7±0.8</td>
<td>0.512</td>
</tr>
<tr>
<td>RDW,%</td>
<td>17.2±2.1</td>
<td>19±2</td>
<td>14.9±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ca-125</td>
<td>28.2±5.5</td>
<td>103±6.9</td>
<td>27.9±4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.2±1.5</td>
<td>11.2±1.4</td>
<td>11.6±1.6</td>
<td>0.403</td>
</tr>
<tr>
<td>Platelet (x10^3 µl)</td>
<td>260±79</td>
<td>289±101</td>
<td>264±78</td>
<td>0.509</td>
</tr>
<tr>
<td>WBC (x10^3 µl)</td>
<td>8.4±1.6</td>
<td>8.8±2.4</td>
<td>8.2±1.9</td>
<td>0.482</td>
</tr>
</tbody>
</table>

RDW: red cell distribution width; WBC: white blood cell.

Figure 1. — Comparison of CA-125 levels in the control and endometriosis subgroups.

Figure 2. — Comparison of RDW levels in the control and endometriosis subgroups.

Figure 3. — The correlation of RDW and CA-125 in the whole population.
Discussion

The present study results indicate that endometriosis patients had significantly increased RDW levels when compared to the control group. Furthermore, RDW levels were associated with the severity of endometriosis. This study documents, for the first time in the literature, that RDW was associated with both presence and severity of endometriosis. Although RDW was initially used as a parameter in the diagnosis of anemia, recently it has been shown to be related to long-term adverse effects in cases of acute and chronic events such as acute myocardial infarction and heart failure and in healthy individuals [11, 13-15]. Tonelli et al. hypothesized that as a cause of cardiovascular diseases, chronic inflammation might lead to elevated RDW [11]. Lippi et al. also revealed that RDW had a significant correlation with hsCRP and sedimentation which is supportive of the mentioned hypothesis [16]. Accordingly, in the present study, the authors observed increased RDW levels in endometriosis, a chronic inflammatory disease.

Although endometriosis is a common gynecologic disease, the exact underlying etiopathogenesis is not yet clearly known. Many hypotheses have been proposed to explain the etiology. First, retrograde menstruation theory gained recognition [17]. However in a study, while 90% of women exhibited retrograde menstruation, only a small percent exhibited endometriosis [18], suggesting that factors other than retrograde menstruation may be involved, as well. Recently, particularly inflammation is investigated in this regard. Cytokine profile and active macrophages in the peritoneal fluid have been shown to rise in endometriosis [19]. Moreover, peritoneal macrophage migration inhibitory factor [20], TNF-α alpha [21], IL-1 BETA, IL-6 [7], and IL-8 have also been observed to be increased in endometriosis. HsCRP, an inflammatory marker, has been shown to be increased in endometriosis patients, as well [22]. Nonetheless, CA-125 is the most commonly studied marker despite its low sensitivity. In the present study, CA-125 level was found to be increased in endometriosis cases, while being positively correlated to RDW.

The association between RDW and endometriosis can be explained with the increased inflammatory markers in endometriosis. Previous studies have shown elevated inflammatory markers in endometriosis patients and also a close relationship between RDW and inflammation [23]. Inflammation increases RDW levels by reducing the life span of erythrocytes via impairing iron metabolism and response to erythropoietin [24]. Moreover, RDW has been shown to have a strong graded relationship with hsCRP and sedimentation [16].

There is a need for further large scale studies in order to completely understand the pathophysiologic relationship between RDW and endometriosis. Nonetheless, the present authors believe that activation of inflammation, which is thought to be closely related to endometriosis, increases RDW levels through its impact on erythropoiesis.

Limitations

One of the most important limitations of the present study was the small number of patients enrolled. Second, since this study was of cross-sectional design, the prognostic value of RDW in endometriosis patients was not investigated. Moreover, lack of data concerning the exact day of menstruation (CA-125 levels are higher in the proliferative phase) [25] may be regarded as the limitations of this study.

References


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Influence of aggressive nutritional support on growth and development of very low birth weight infants

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Department of Neonatology, Soochow University Affiliated Children’s Hospital, Suzhou, Jiangsu Province, (China)

Summary
Aim: To investigate the influence of the early postnatal aggressive nutritional support on the very low birth weight infants (VLBWI) during hospitalization. Materials and Methods: Surviving premature infants without obvious deformity, with gestational age more than 28 weeks and less than 32 weeks, birth weight 1,000 g to 1,500 g, admitted in NICU in Affiliated Children’s Hospital of Suzhou University during 12 hours after birth and stay for two weeks or more from January 2008 to December 2011 were selected, including 44 cases (admitted from September 2010 to December 2011) in the observation group and 36 cases in the control group (admitted from January 2008 and September 2010). The infants in the observation group were treated by aggressive nutritional management, while traditional nutritional management for infants in the control group. The variations of nutritional intake, weight gain, jaundice index, blood biochemistry, serum electrolytes indexes, and complications were compared between the two groups. Results: Compared to the control group, the average growth rate and the albumin (ALB) and prealbumin (PA) levels two week after birth and before leaving hospital of the infants in the observation group was significantly higher (p<0.05), and the incidence of the extrauterine growth retardation was significantly decreasing (p<0.05). However, the days of hyperbilirubinemia, highest value of the serum bilirubin, duration of jaundice, platelets after intravenous nutrition, liver function, blood lipid levels, blood glucose, blood PH, serum creatinine, urea nitrogen, and electrolytes of the first day and the seventh day after birth and the incidence of parenteral nutrition-associated cholestasis (PNAC) and necrotizing enterocolitis (NEC) between the two group had no difference (p>0.05). Conclusion: The implementation of aggressive nutritional management on the with VLBWI was safe and effective.

Key words: Aggressive nutritional support; Very low birth weight infants; Enteral nutrition; Parenteral nutrition.

Introduction
Currently, the global incidence of preterm infants is 9.6% [1], including 22.5% low birth weight infant (LBWI) [2]. With the gradual improvement of the perinatal medicine and neonatal intensive care treatment technology, the survival rate of the VLBWI and the super low birth weight infant increases year by year and reaches 66.6% - 84% [3-5]. Subsequently, the situation for growth deficiency of the postnatal VLBWI is becoming more common, and extrauterine growth retardation (EUGR) occurs in some VLBWI. According to the reported clinical and experimental data, EUGR has irreversible long-term effects on the neural development of the future and the final height [1, 6, 7], and increases the liability risk of the cardiovascular disease and metabolic syndrome in adult [8, 9]. Therefore, formulating rational nutritional support programs to promote the growth and development of the VLBWI has become the focus work of NICU. Many previous researches are about early nutritional support for preterm infants, but recent studies show that the growth rate of the VLBWI from birth to 18-24 months of the corrected age is related to the outcomes of the neural development [7], but how about the efficacy and safety issues of the aggressive enteral and parenteral nutritional support in the entire hospital stay? Thus, the clinical data of the VLBWI hospitalized from January 2008 to December 2011 were retrospectively analyzed to discuss the influence on the parenteral and enteral nutritional support of the VLBWI during hospital stay.

Materials and Methods
Study objects and grouping
Forty-four VLBWI admitted in neonatal NICU of the Affiliated Children’s Hospital of Suzhou University from September 2010 to December 2011 were taken as the observation group, including 25 males and 19 females with mean gestational age 30.32 ± 1.360 weeks, mean birth weight 1,275.45 ± 114.434 g. Thirty-six VLBWI admitted in neonatal NICU of the Affiliated Children’s Hospital of Suzhou University from January 2008 to September, 2010 were taken as the control group, including 22 males and 14 females with mean gestational age 30.67 ± 1.512 weeks, mean birth weight 1,303.08 ± 165.004 g. There were no significant difference in the rate of the gender, infants under gestational age, asphyxia, neonatal respiratory distress syndrome, birth weight and gestational age (p>0.05) (Table1). This study was conducted in accordance with the declaration of Helsinki, and with approval from the Ethics Committee of Soochow University Affiliated Children’s Hospital. Written informed consent was obtained from all participants.

Inclusion criteria: (1) Premature infants with gestational age <32 weeks and ≥ 28 weeks, birth weight < 1,500 g and ≥ 1,000 g; (2) admission time < 12 hours and hospital stay ≥ two weeks; (3) steady vital signs, no cyanoderma, no apnea, completely oral feeding when left hospital, and (4) with complete medical history and clinical data.

Exclusion criteria: (1) Infants with congenital metabolic diseases, severe congenital heart disease and gastrointestinal malformations, and (2) infants with hospital stay < two weeks, poor sucking when left hospital or unstable vital signs.
Table 1. — Comparison of general data for VLBWI between the two groups when admitted

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Male/female (cases)</th>
<th>Infants under gestational age (%)</th>
<th>Asphyxia (%)</th>
<th>Respiratory distress syndrome of infants (RDS) (%)</th>
<th>Average gestational age (weeks)</th>
<th>Birth weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>44</td>
<td>25/19</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>30.32±1.360</td>
<td>12754.5±1144.346</td>
</tr>
<tr>
<td>Control group</td>
<td>36</td>
<td>22/14</td>
<td>13</td>
<td>9</td>
<td>6</td>
<td>30.67±1.512</td>
<td>1303.08±165.004</td>
</tr>
<tr>
<td>χ² value</td>
<td></td>
<td>0.092</td>
<td>0.21</td>
<td>1.347</td>
<td>2.033</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t value</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1.08</td>
<td>-0.882</td>
</tr>
<tr>
<td>P value</td>
<td>0.762</td>
<td>0.886</td>
<td>0.246</td>
<td>0.154</td>
<td>0.282</td>
<td>0.381</td>
<td></td>
</tr>
</tbody>
</table>

Methods

Retrospective controlled study was used to collect the situation of the selected objects such as births, enteral and parenteral nutrition, weight gain, blood biochemistry, and physical development.

Observation group: The VLBWI of the observation group was treated by early aggressive enteral and parenteral nutritional management as follows: (1) parenteral nutrition: all cases admitted to hospital within 24 hours were treated by intravenous nutrition. Composition of intravenous nutrition: glucose: initial amount was four to six mg/(kg.min), increased one to two mg/(kg.min) for each day if tolerated until reaching requisite amount, the maximum was up to 13 - 17 g/(kg.d). Amino acids: amino acids were given within four hours or at least within 12 hours after birth, the initial amount was 2.0 g/(kg.d), reached 4.0 g/(kg.d) at the velocity of 0.5 - 1.0 g/(kg.d). Fat milk: fat milk was given within 24 hours after birth, the initial amount was 0.5 - 1.0 g/(kg.d), reached 3.0 g/(kg.d) at the velocity of 0.5 - 1.0 g/(kg.d), and stopped intravenous nutrition when the enteral nutrition reached 100 k cal/(kg.d). Bilirubin values and liver function were regular monitored, the fat milk was stopped being given if the bilirubin value was too high or the liver function was impaired. (2) Enteral nutrition: shield water milk (energy: 81 k cal/100 ml, protein: 2.2 g/100 ml) was used. Usually, the minimal enteral feeding (MEF) was given the day after birth, or at least five days after birth for the infants with serious condition if tolerated, increased 10-20 ml/(kg.d) for each day, and transited to full enteral feeding as soon as possible. Feeding was stopped or just restricting to MEF if intolerance appeared during feeding. Feeding patterns: oral feeding firstly, or tube feeding (usually interval feeding) if uncoordinated swallowing condition occurred in the infants or conditions did not allow oral feeding, then gradually transiting to oral feeding.

Control group: the VLBWI of the control group were treated by traditional nutritional management as follows: (1) parenteral nutrition: composition of intravenous nutrition: Glucose: initial amount was four to six mg/(kg.min), increased one to two mg/(kg.min) for each day if tolerated until reaching requisite amount, the maximum was up to 13 - 17 g/(kg.d). Amino acids: amino acids were given within 24-48 hours after birth, the initial amount was 0.5 g/(kg.d), reached 3.0 - 3.5 g/(kg.d) at the velocity of 0.5 - 1.0 g/(kg.d). Fat milk: fat milk was given three days after birth, the initial amount was 0.5 g/(kg.d), reached 3.0 g/(kg.d) at the velocity of 0.5 g/(kg.d). Bilirubin values and liver function were regular monitored, the fat milk was stopped to be given if the bilirubin value was too high or the liver function was impaired. (2) Enteral nutrition: shield water milk (energy: 81 k cal/100 ml, protein: 2.2 g/100ml) was used. Usually, the MEF was given five days after birth if tolerated, increasing 5-10 ml/(kg.d) for each day, and transiting to full enteral feeding as soon as possible. For infants with the assistance of breathing machine or CPAP machine, began MEF after removing the machines. Feeding patterns: oral feeding firstly, or tube feeding (usually interval feeding) if uncoordinated swallowing condition occurred in the infants or conditions did not allow oral feeding, then gradually transiting to oral feeding.

Observing indexes

(1) Observing and comparing the albumin (ALB) and prealbumin (PA) levels of the day, two weeks after birth, before leaving hospital, the time for regaining birth weight, the rate of gaining weight, the time of reaching full enteral feeding (PN time), neonatal necrotizing enterocolitis (NEC), parenteral nutrition associated cholestasis (PNAC) and the incidence of EUGR when left hospital. (2) Observing and comparing the days of hyperbilirubinemia occurring and duration of jaundice and the detection of platelet count (PLT) after parenteral nutrition (PN), the total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT), and total bilirubin (TB). (3) Observing and comparing the values such as serum creatinine, urea nitrogen, pH, and electrolytes of the first day and the seventh day after birth.

Related diagnostic criteria

(1) Hyperbilirubinemia: The VLBWI or infants with other risk factors were more susceptible to bilirubin encephalopathy, and there was risk of bilirubin encephalopathy when the TB reached 171.0 - 205.2 μmol/l [10]. Therefore, the hyperbilirubinemia in this study was defined as > 171 μmol/l. (2) Days of hyperbilirubinemia: the days of the serum total bilirubin > 171 μmol/l. (3) Duration of jaundice: period between the start of yellow skin and the end of the jaundice vanishing. (4) PNAC: application time of parenteral nutrition ≥ two weeks, and serum direct bilirubin ≥ 25.6 μmol/l. (5) EUGR: the growth and development indexes were above or under the 10th percentile of the expected values for the corresponding intrauterine growth rate [11].

Statistical analysis

Data were analyzed using SPSS17.0 statistical software. Measurement data in line with normal values were indicated as ±s, the measurement data between the two groups were compared using t test, enumeration data between the two groups were compared using χ² test, and p < 0.05 indicated that the differences had no statistical significance.

Results

Comparison of general data for VLBWI between the two groups when admitted

There were no significant differences in the number of cases, gender, gestational age, birth weight, the incidence of infants under gestational age, and the fundamental disease between the two groups (p > 0.05, Table 1).

Comparison of nutritional reserves and nutritional biochemical indexes between the two groups

The differences of the time for regaining birth weight and the time for reaching full enteral feeding between the two
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groups had no statistical significance (p > 0.05), but the time was shorter in the observation group than that in the control group. The rate of gaining weight in the observation group was obviously faster than that in the control group, the difference had statistical significance (p < 0.05). The differences of ABL and PA levels between the two groups when admitted had no statistical significance (p > 0.05), but the difference of ABL and PA levels in the observation group was obviously higher than that in the control group two weeks after birth and before leaving hospital, suggesting the difference had statistical significance (p < 0.05) (Table 2).

Comparison of the jaundice and the biochemical indices after PN between the two groups

There were no significant differences in the days of hyperbilirubinemia occurring, the highest value of serum bilirubin and the duration of jaundice between the two groups (p > 0.05), but the difference of ABL and PA levels in the observation group was obviously higher than that in the control group two weeks after birth and before leaving hospital, suggesting the difference had statistical significance (p < 0.05) (Table 2).

Comparison of experimental indexes such as acid-base balance and electrolyte between the two groups

There were no significant differences in the days of hyperbilirubinemia occurring, the highest value of serum bilirubin and the duration of jaundice between the two groups (p > 0.05). The differences of the PLT after PN, the liver function and the blood lipid levels between the two groups had no statistical significance (p > 0.05, Table 3).

Discussion

EUGR was a major problem for worldwide VLBWI. Preterm infants had to go through the process of catch-up growth after birth, but the time was very short. The most rapid speed for catching up was three months after birth, and the critical growing period for head circumference was only the first year after birth. If there was no catch-up growth in the critical period, the chance for later was very limited so that the neural development and the final

Table 2. — Comparison of nutritional reserves and nutritional biochemical indexes between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Velocity of gaining weight (g/kg/d)</th>
<th>Time for oral feeding (d)</th>
<th>Time for regaining birth weight (d)</th>
<th>Time for reaching full length intestine feeding (d)</th>
<th>The day after birth (ABL mg/l)</th>
<th>Two weeks after birth (ABL mg/l)</th>
<th>Leaving hospital (ABL mg/l)</th>
<th>ABL (g/l)</th>
<th>PA (g/l)</th>
<th>ABL (g/l)</th>
<th>PA (g/l)</th>
<th>ABL (g/l)</th>
<th>PA (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>44</td>
<td>21.80±2.952 2.14±1.391 8.23±5.794</td>
<td>27.73±11.050</td>
<td>32.30±70.43±</td>
<td>34.55±93.09±</td>
<td>35.45±104.75±</td>
<td>3.470 18.918</td>
<td>2.090 18.632</td>
<td>2.006 34.644</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.027</td>
<td>0.241</td>
<td>0.096 0.233</td>
<td>0.000 0.004</td>
<td>0.000 0.000</td>
<td>0.000 0.000</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 3. — Comparison of the jaundice and the biochemical indices after PN between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Days of hyperbilirubinemia occurring (d)</th>
<th>The highest value of serum bilirubin (mg/dl)</th>
<th>Duration of jaundice (d)</th>
<th>PLT (10^11 L)</th>
<th>CHOL (mmol/L)</th>
<th>TG (mmol/l)</th>
<th>ALT (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>44</td>
<td>3.97±0.77</td>
<td>13.93±2.292</td>
<td>9.30±4.448</td>
<td>231.67±79.532</td>
<td>2.45±0.952</td>
<td>0.91±0.67</td>
<td>13.52±14.117</td>
</tr>
<tr>
<td>Control group</td>
<td>36</td>
<td>3.58±1.301</td>
<td>14.55±5.235</td>
<td>9.72±7.067</td>
<td>205.58±81.187</td>
<td>2.07±1.068</td>
<td>0.70±0.419</td>
<td>12.66±10.921</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.154</td>
<td>0.543</td>
<td>0.789</td>
<td>0.220</td>
<td>0.159</td>
<td>0.170</td>
<td>0.792</td>
</tr>
</tbody>
</table>

Table 4. — Comparison of experimental indexes such as acid-base balance and electrolyte between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>pH  The first</th>
<th>The seventh</th>
<th>BUN The first</th>
<th>The seventh</th>
<th>CR The first</th>
<th>The seventh</th>
<th>The first</th>
<th>The seventh</th>
<th>The first</th>
<th>The seventh</th>
<th>The first</th>
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<th>The first</th>
<th>The seventh</th>
<th>The first</th>
<th>The seventh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>44</td>
<td>7.33±0.335</td>
<td>7.35±0.335</td>
<td>3.03±4.78±</td>
<td>50.2±66.5</td>
<td></td>
<td>4.1±4.1</td>
<td></td>
<td>5.4±5.4</td>
<td></td>
<td>138.6±140.3</td>
<td></td>
<td>4.7±4.7</td>
<td></td>
<td>5.1±5.1</td>
<td></td>
<td>96.3±97.4</td>
<td></td>
<td>5.1±5.1</td>
<td></td>
<td>6.3±6.3</td>
</tr>
<tr>
<td>Control group</td>
<td>36</td>
<td>7.34±0.360</td>
<td>7.36±0.360</td>
<td>3.75±4.35±</td>
<td>49.5±66.9</td>
<td></td>
<td>4.3±4.3</td>
<td></td>
<td>5.2±5.2</td>
<td></td>
<td>139.2±140.8</td>
<td></td>
<td>4.6±4.6</td>
<td></td>
<td>5.0±5.0</td>
<td></td>
<td>96.6±97.3</td>
<td></td>
<td>5.0±5.0</td>
<td></td>
<td>6.7±6.7</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.767 0.887</td>
<td>0.259 0.423</td>
<td>0.832 0.872</td>
<td>0.542 0.431</td>
<td>0.654 0.893</td>
<td>0.753 0.932</td>
<td>0.931 0.899</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 5. — Comparison of hospital stay and complications between the two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Hospital stay (days)</th>
<th>EUGR n (%)</th>
<th>PNAC n (%)</th>
<th>NEC n (%)</th>
<th>Septicemia n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>44</td>
<td>43.93±9.978</td>
<td>19 (43.2)</td>
<td>4 (9.1)</td>
<td>3 (6.8)</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td>Control group</td>
<td>36</td>
<td>51.03±16.636</td>
<td>27 (75)</td>
<td>6 (16.7)</td>
<td>4 (11.1)</td>
<td>3 (8.5)</td>
</tr>
</tbody>
</table>

| χ² value          | -     | 8.203                | 1.03       | 0.457      | 0.485     |                  |
| t value           | 2.36  | -                    | -          | -          | -         |                  |
| p value           | 0.021 | 0.004                | 0.308      | 0.499      | 0.486     |                  |

It was reported that the nutritional deficiency of the VLBWI and the low birth weight children after birth could be reduced due to the early aggressive nutrition support (including enteral and parenteral nutrition) [12]. Giving nutritional support with high energy and high protein for the VLBWI could reduce the incidence of EUGR [13, 14], and the safety of high-protein nutritional support in the early days after birth was explicit [15, 16]. Therefore, the early aggressive enteral and parenteral nutrition support were advocated both at home and abroad to promote the early postnatal catch-up growth and reduce the incidence of EUGR [17]. The results of the retrospectively analysis for the clinical data of the VLBWI treated by aggressive nutrition support in the entire hospital stay showed that the growth rate of the average weight for the observation group was significantly shorter than that for the control group, but the hospital stay was longer. Although the time of regaining birth weight and the time of reaching full length-intestine nutrition in the observation group were shorter than that in the control group, but the difference had no statistical significance. Inconsistent with the report in the foreign literature which confirmed the aggressive nutritional management could shorten the time to regain birth weight [18], the results in this study might be related to the less than normal number of cases, so further in-depth study should be performed in the future. Serum ABL with half-life of 25-26 days and serum PA with half-life of only 1.9 days were mainly synthesized by the liver. The changes of the ALB and PA due to the changes of protein and energy in vivo would provide evaluated indicators for nutritional reserves of the premature infants. It was generally believed that the early postnatal nutritional status could be reflected by the values of the first two weeks after birth, and the values before leaving hospital reflected the overall nutritional status during hospitalization [19]. The results in this study showed that the ABL and PA levels of the observation group two weeks after birth and before leaving hospital were significantly higher compared with that of the control group, indicating that the aggressive nutritional management could significantly increase the body’s nutritional reserves of the VLBWI. The incidence of EUGR in the observation group was significantly decreased, indicating that aggressive nutritional management could promote early growth and development of the VLBWI and reduce the incidence of EUGR. This nutritional management was effective, but how about its security?

It was known that the natural energy reserves of the VLBWI were limited. According to Heird [20], the VLBWI could only survive for four days only depended on their natural energy reserves without absorbing exogenous energy. Certainly, the quick intake of the carbohydrate could be used to replenish the lost energy, but this would lead to negative nitrogen balance and self-protein deficiency of the VLBWI, which happened in the early stage would affected their neural development of the VLBWI. At least 1.5 g/kg of proteins were given daily in order to achieve a positive nitrogen balance. Therefore, the current domestic and international research recommended amino acid giving within a few hours after birth, the initial amount was 2.0 g/(kg.d), gradually reaching 0.5 - 1.0 g/(kg.d) at a velocity of 4.0 g/(kg.d). However due to the early studies suggested that high doses of amino acids to VLBWI could cause metabolic acidosis, renal dysfunction, and other diseases [21], many clinicians currently still followed the traditional nutrition policies. In this study, compared with the control group, the early postnatal supply of amino acids did not increase metabolic acidosis, renal dysfunction, electrolyte imbalance, and other disease in the observation group, which was in line with the study of Thureen et al. [22], indicating that the application of early aggressive clinical amino acid was safe.

The early postnatal enteral nutrition of the VLBWI could not meet their caloric needs, therefore the supply of the energy mainly relies on fat milk. Although the optimal amount fat intake constituted for body organization remained unclear, research institutions at home and abroad all recommended to begin giving fat milk within 24 hours after birth, the initial amount was 2.0 g/(kg.d), and reaching 3.0 g/(kg.d) at the velocity of 0.5-1.0 g/(kg.d). However, since the systems of the preterm infants were immature, early application of adequate fat milk to preterm infants could aggravate neonatal jaundice, cause hyperlipidemia, cholestasis, liver damage, and other diseases [23]. In addition, the current clinical application of soy-derived fat milk was rich in n-6 fatty acids, which had proinflammatory mechanisms and could promote hepatic apoptosis and necrosis [23, 24], and arachidonic acid (one of the derivatives for n-6 fatty acids, which had proinflammatory mechanisms and could promote hepatic apoptosis and necrosis [23, 24], and arachidonic acid (one of the derivatives for n-6 fatty acids, which had proinflammatory mechanisms and could promote hepatic apoptosis and necrosis [23, 24].
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acids) could lead to dysfunction, adhesion reduction, and quantitative reduction of the platelet, which manifested bleeding in clinics [24]. Therefore, at present, many clinicians still tended to use the traditional nutrition programs, that is, beginning giving fat milk from the third day after birth, slowly increasing the dose and reaching a sufficient amount on the sixth day after birth. At least 0.5 \( g\cdot kg^{-1}\cdot d^{-1}\) fat milk was needed for preterm infants to prevent the essential fatty acid deficiency [25], but many clinicians often applied a higher dose fat milk to meet the need of infant growth. Long-term parenteral nutrition was not needed by most preterm infants in the NICU, so the minimum amount of fat milk strategy was not necessary for most infants [23, 26]. In this study, fat milk was given 24 hours after birth, but the days of hyperbilirubinemia occurring, the highest value of serum bilirubin, the duration of jaundice, and the PLT, liver function, serum lipids, incidence of PNAC after PN of the observation group had no significantly increasing compared with that of the control group. Visibly, early application of fat milk did not increase the risk of bleeding, jaundice, liver dysfunction, and cholestasis in the VLBWI, which was consistent with the findings of Ibrahim et al. [27], indicating that the aggressive application of fat milk nutrition during hospitalization for the VLBWI was safe.

It was conventionally believed that too early initiation of enteral nutrition could lead to an increased incidence of NEC, therefore, more clinicians currently tended to commence intestinal feeding rather later. However, studies showed that early initiation of enteral feeding, such as beginning receiving parenteral MEF five days after birth did not increase the incidence of NEC [28, 29]. In this study, the average time for starting milk feeding was 2.14 days after birth for the observation group, and 6.67 days for the control group; the incidence of NEC between the groups had no significant differences, indicating that accepting intestinal feeding after birth for the VLBWI did not increase the incidence of NEC.

This study suggested that giving aggressive vein and intestinal feeding for the VLBWI during hospitalization was safe and effective, which deserved more clinicians promoting and researching in order to better promote the growth and development of the VLBWI and improve long-term prognosis. The present also need follow-up studies for further long-term neural development.

Acknowledgements

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Marked improvement of vulvovaginitis of unknown origin in a pediatric patient - case report

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Summary

Purpose: To present a novel therapy for pediatric vulvovaginitis. Materials and Methods: An eight-year-old girl with persistent severe vulvovaginitis of unknown origin also complained of unexplained weight gain and sudden academic difficulties. She was treated with dextroamphetamine sulfate. Results: She not only showed very quick and excellent relief from her vulvovaginitis but she also lost weight and improved her mentality. Conclusions: Sympathomimetic amine therapy may benefit pediatric vulvovaginitis when an infectious cause cannot be ascertained.

Key words: Pediatric vulvovaginitis; Sympathomimetic amines; Sympathetic nervous system hypofunction.

Introduction

Hypofunction of the sympathetic nervous system has been linked to a wide variety of chronic disorders refractory to standard therapy but very responsive to treatment with sympathomimetic amines especially dextroamphetamine sulfate [1, 2]. Various pelvic pain syndromes especially chronic pelvic pain, dysmenorrhea, dyspareunia, and interstitial cystitis respond quickly and effectively to dextroamphetamine sulfate despite failing other therapies [3, 4]. Vulvovaginitis is another part of the chronic pain syndrome which in the author’s experience sometimes responds to dextroamphetamine sulfate but not as well as other types of pelvic pain.

The case presented herein describes an enigmatic case of severe vulvovaginitis in an eight-year-old that responded very well to dextroamphetamine.

Case Report

A mother brought her eight-year-old daughter to the authors’ reproductive endocrine group with a frustrating problem. Though our group generally does not see pediatric patients the mother was frustrated by the failure of multiple specialists including pediatric endocrinologists, pediatric gynecologists, infectious disease specialists, and neurologists to determine the etiology of her daughter’s sudden development of weight gain, becoming a student who struggled academically when she was previously the top of her class, and her development of a malodorous vaginal discharge and a very painful vulvovaginitis. Also she started very early pubarche at age seven.

Laboratory tests taken at age nine found her electrolytes to be normal except the potassium was somewhat low at 3.6 mmol/L (normal 4.1–5.8 mmol/L). The rest of the complete metabolic panel was normal. Serum iron was normal. Her complete blood count was normal as was her sedimentation rate of 7 (0–20 mm/hr).

Conclusions: She not only showed very quick and excellent relief from her vulvovaginitis but she also lost weight and improved her mentality. Conclusions: Sympathomimetic amine therapy may benefit pediatric vulvovaginitis when an infectious cause cannot be ascertained.
Jersey where she was first seen so they could prescribe dextroamphetamine sulfate without the affirmed diagnosis of ADHD (and thus considered an off-label use) because in New Jersey it is not legal to prescribe any class II drug off-label.

Within a very short length of time the young lady who was now nine-years-old showed tremendous improvement following treatment with dextroamphetamine sulfate extended release capsules 15 mg once daily. She began losing weight and her mentality improved to her former top student self. Also, and the subject of this case report, her severe vulvovaginitis completely cleared up. Interestingly the slightly malodorous mysterious intermittent vaginal discharge still occurred but was not associated with vaginal irritation. Previously, though, the young girl stated that she had episodes of introital irritation without the discharge but she believed that the vaginal discharge made it worse; now the vaginal discharge causes no irritation at all.

**Discussion**

The authors have hypothesized that one common factor, i.e., hypofunction of the sympathetic nervous system, is a frequent main etiologic factor in a wide variety of chronic disorders that are refractory to standard therapy or perhaps show some improvement with standard therapy but have a risk of serious side effects and/or life-threatening complications with these standard therapies [1, 2]. But most importantly these various disorders respond very well to a very well-tolerated medication with no long term sequelae, i.e., dextroamphetamine sulfate [1, 2]. The reason for the class II label is when used in illicit not pharmacological dosages it can be addicting.

Dextroamphetamine sulfate to treat edema has been used for more than 50 years [6]. More than 25 years ago the authors demonstrated that severe long term treatment resistant urticaria improved markedly following sympathomimetic amine therapy probably related to reducing the permeability from histamine containing vesicles [7].

With prior publications on the use of sympathomimetic amines for medical conditions compounded with the fact that dextroamphetamine sulfate is available as a generic and it is a class II drug makes it highly unlikely that a pharmaceutical company will promote this medication.

Without the likelihood of the support from pharmaceutical companies the main way to promulgate the knowledge is by publications. Thus whenever a new unique presentation of this syndrome occurs, e.g., vulvovaginitis in an eight-year-old that responds to sympathomimetic amine therapy, the authors will attempt to publish the case report. Unfortunately for unexplained reasons most physicians seem to be resistant to this concept and simple safe therapy. The authors published a case report of a woman with Crohn’s disease Stage IV with 8-12 painful bowel movements who failed to improve despite cyclophosphamide, glucocorticoids, and three different types of anti-tumor necrosis alpha inhibiting drugs and was advised by a highly regarded gastroenterologist who specialized in Crohn’s disease that she needed a colectomy and ileostomy [8]. Despite the fact that she was 90% improved within one week of taking the dextroamphetamine sulfate 15 mg extended release capsules and 100% improved (one painless bowel movement per day) within one month of therapy and repeat colonoscopy showing complete remission, her treating physician strongly believed that the remission was unrelated to medication. The patient’s gastroenterologist believed her recovery was merely spontaneous and in the future does not plan on using it on any other patients with Crohn’s disease [8].

Another interesting anecdote was a case the authors have not reported where a woman had 20 years of severe migraines that her consulting neurologist was unable to ever find one drug combination to give her relief, but his belief was that one day she would develop multiple sclerosis. She had immediate relief from taking dextroamphetamine sulfate and her migraines which were daily completely disappeared. When she returned to her neurologist he advised her to stop the medication saying it was a dangerous drug, but on cessation her migraines returned immediately. She restarted her dextroamphetamine sulfate and her symptoms and her migraines completely abated again.

The authors believe that this is the first report of treating a case of vulvovaginitis in the pediatric population with dextroamphetamine sulfate. It is believed that the sympathomimetic amine’s benefit was related to diminishing cellular permeability which not only allowed absorption of irritating chemicals from sweat and from the vaginal discharge into the vulva or vagina, but also diminished capillary permeability when standing which had led to edema and weight gain she did return to her normal weight after treatment [5].

**References**


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Intraoperative asystole associated with fibroid uterus

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Summary
The authors present the case of a patient with a large retroperitoneal fibroid whose laparotomy was abandoned due to intraoperative asystole. Perioperative management and possible etiological factors are discussed in this paper.

Key words: Retroperitoneal fibroid; Asystole; Uterus.

Introduction
Intraoperative asystole during benign gynaecological surgery is a rare event. The authors present a patient whose prior procedure was abandoned due to asystole of unknown etiology. The pre- and intraoperative management of such a patient requires careful planning.

Case Report
A 47-year-old para two attended a gynaecologist in a secondary centre complaining of menorrhagia. An ultrasound suggested a 15-cm uterine fibroid and a decision was made for the patient to undergo laparotomy with either myomectomy or hysterectomy. This patient had no significant past medical history and no surgical history of significance.

Intraoperatively a large retroperitoneal pelvic mass was identified. This appeared to be separate from the uterus and ovaries. Upon manipulation of the mass, the patient developed asystole. The attending anaesthetist resuscitated the patient using atropine, such that both the heart rate and blood pressure returned to within normal ranges. Once the patient was stabilised, the assistance of a general surgeon was sought. Prior to gaining surgical access to the retroperitoneum, the patient again developed asystole once the mass was palpated. The patient was again stabilised and the procedure abandoned. Referral was sent to the tertiary gynae-oncology centre following an uneventful recovery.

At clinical review, possible etiologies including cardiac dysrhythmia or the presence of phaeochromocytoma or neuroblastoma were explored. Preoperative investigations included computerised tomography scans and magnetic resonance imaging of the abdomen and pelvis both of which demonstrated a large pelvic mass with the appearances of a leiomyoma. No other masses were present and there was no appreciable lymphadenopathy. Urinary catecholamines and metanephrines were not present. Electrocardiogram was normal. The patient was reviewed by the anaesthetic team and a decision was made that during repeat laparotomy, external pacing pads would be placed prior to induction of general anaesthesia.

Repeat laparotomy demonstrated a large pelvic mass which originated from the uterine cervix. It had invaded the retroperitoneal space and entered the para-rectal space, inferior to the sigmoid mesentery. Upon opening the abdomen, the patient became severely bradycardic and hypotensive which responded to two separate doses of atropine 0.5 mg given intravenously. A total abdominal hysterectomy was performed and the leiomyoma was removed in its entirety (Figure 1).

The patient had an uneventful recovery and was reviewed postoperatively by cardiology. Investigations included further electrocardiogram and a cardiac stress test, both of which were unremarkable. Histological assessment concluded that the leiomyoma was entirely benign in nature and the uterus was unremarkable.

Discussion
The development of asystole during hysterectomy or myomectomy is very rare. A comprehensive literature search, using PubMed and Medline, was performed and few papers describing this event were found.

One possible etiological factor is vagal stimulation during dissection of the retroperitoneum, leading to severe bradycardia and hypotension. Although blood pressure may decrease while the retroperitoneum is accessed, asystole is an extremely rare event. Atropine is employed to prevent and treat this complication.

Figure 1. — Uterus, tubes, and cervical fibroid.
A further possible cause is sick sinus syndrome. This has been described in one case report as having occurred during hysterectomy [1]. Arrhythmias, including severe bradycardia, may occur intraoperatively in patients with this syndrome and atropine is employed as part of their management.

Vasopressin is commonly used as an adjunct to reduce blood loss during resection of leiomyomae. It has been described in two separate reports [2, 3] as leading to cardiac arrest. In both of the surgical procedures that this patient underwent, vasopressin was not used.

Rarely, leiomyomae or leiomyosarcomae [4] may undergo intravascular spread and their growth can reach the right atrium, leading to cardiac complications. This patient had no such intravascular spread and the leiomyoma did not undergo sarcomatous change.

Conclusions

This case highlights the need for awareness of the management of severe bradycardia or asystole encountered in retroperitoneal gynaecological surgery. The authors recommend early and judicious use of atropine, the use of external pacing pads, and gentle handling of retroperitoneal masses. They also recommend consideration of possible etiologies including sick sinus syndrome and vagal stimulation.

References


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Pregnancy and delivery after vesico ileocystoplasty –
a case report

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Summary

Neobladder is continent urinary reservoir made from a detubularized segment of bowel, with implantation of ureters and urethra. The most common indication for this operation is bladder replacement after cystectomy following bladder cancer in elderly or cervical cancer Stage IV patients. Nowadays indications are expanded to many benign diseases (interstitial cystitis (IC), neurogenic bladder, chronic pelvic pain) in reproductive age. Pregnancy in women with neobladder is a rare condition, hence published experience is limited. Most of the published cases were delivered by cesarean, due to the concern for possible complications. The authors report a case of a 36-year-old woman who underwent a vesico ileocystoplasty for IC, became pregnant six years after the operation, and delivered a healthy baby vaginally. Her obstetric and urological outcomes were assessed, during, and after pregnancy. Careful antenatal monitoring by both an obstetrician and a urologist, awareness of potential complications, and their prompt treatment, can result in a successful pregnancy and vaginal delivery where neither fetus or mother nor neobladder are endangered.

Key words: Neobladder; Vesico ileocystoplasty; Pregnancy; Delivery.

Introduction

Interstitial cystitis (IC) is a chronic inflammatory disease of the bladder with unknown etiology characterized by suprapubic pain, urinary frequency, and urgency that renders patients socially incapacitated [1]. Many terms that refer to IC or IC-related conditions have been used: painful bladder syndrome, chronic pelvic pain syndrome, and bladder pain syndrome. Treatment is mostly non-cui-rative because of its yet unknown etiology. Bladder substitution by ileal neobladder for women who suffer from IC can be a satisfactory option after failure of conserva-tive treatment but is the last resort [2, 3]. About 90% of IC patients are women, median age thirty and forty, hence within reproductive age range. Surgical procedure cannot only improve their quality of life, but conse-quently lead to pregnancy desire [3]. In pregnancy, glomerular filtration rate (GFR) and renal plasma flow increase by 40% to 65% and 50% to 85%, respectively, and roughly parallel to change in cardiac function. Marked dilation of the ureters is caused by hormonal influences and pressure from the enlarged uterus on the ureters. On the other hand, reduced absorptive bowel capacity (due to functional loss of those segments required for reservoir construction), its highly unphysiological exposure to urine, and specialized secretory properties, predisposes patients to various metabolic, nutritive, and functional disturbances and complications [4].

Scanty literature available presents rare cases of pregnancy after augmentation cystoplasty, orthotopic neobladder, Indiana pouch and vaginoplasty, gastrocystoplasty, Mitrofanoff procedure, but not after vesico ileocystoplasty [5, 6].

The authors present a case of pregnancy and delivery management after vesico ileocystoplasty, with assessment of the obstetric and urological outcomes during and after pregnancy.

Case Report

A 36-year-old woman was diagnosed IC by transurethral resection and biopsy of bladder, and she underwent surgical treatment with bladder reconstruction. Since the operation was complicated with post-surgical stenosis of the bladder neck and urethra, she was put on clean intermittent self-catheterization instead of self-catheterization procedure every three hours. Laboratory test showed no ane-mia, nor electrolyte or renal disturbances and no pathological bacterial growth in urine culture. Cervical and vaginal swabs revealed Candida spp. Her serum urea, creatinine, and elec-trolyte levels were followed up every three to four weeks and ultrasonography weekly. At the third day of hospitalization, painful sensation progressed despite bed rest and gestagen ther-apy. Transvaginal sonography revealed further cervical shortening to six mm. The authors administered concomitantly...
dexamethasone six mg/12 hours for two days, for fetal lung maturation, with parenteral β2 adrenergic agonist therapy. Interdisciplinary team (obstetricians, urologist) made a decision about expectative management, continuation of gestation therapy, along with intensive maternal and fetoplacental unit monitoring. Signs of preterm delivery withdraw. During hospitalization, she had three episodes of febrile asymptomatic bacteriuria (Escherichia Coli, Pseudomonas aeruginosa, Morganella morgagni), treated promptly by adequate antimicrobial medications. Although closely observed, neither renal function impairment nor electrolyte imbalance appeared. In 36th week of gestation, obstetric assessment revealed cephalic presentation and cervical effacement with advanced dilatation. Serum urea, creatinine, uric acid, total proteins, albumins, sodium, potassium, chloride and calcium were within the normal range. Diuresis was 2,700 ml/24 hours, with slightly increased proteinuria (0.49 g in 24-hour urine collection), and normal urea and creatinine clearances. Urologist and obstetrician made interdisciplinary decision about spontaneous vaginal delivery with constant indwelling catheter during delivery and seven post-delivery days. She gave birth to a term, healthy child, body weight 3,200 g. After delivery, the authors started antibiotic prophylaxis with broad spectrum antibiotic. Post-delivery urine culture showed no pathological bacterial growth, and markers of urologic function were normal. Her recovery was uneventful, with levels of inflammation markers typical for puerperium. She was discharged with her baby on the fifth post-delivery day after normal clean intermittent catheterization was resumed.

Discussion

Antenatal care, delivery, and post-delivery care of pregnant patients after ileocystoplasty, must involve obstetrician and urologist with knowledge and awareness of potential complications. These short and long-term complications include: urinary tract infections, electrolyte abnormalities, malabsorption, diarrhea, hypochloremic metabolic acidosis, impaired renal function, continuation of intestinal mucus production, and abnormal drug kinetics. Interdisciplinary approach should provide prevention of those complications when possible, their early detections, and prompt treatment.

Mucus produced in ileocystoplasties has potential influence on upper urinary tract drainage and emptying of the bladder reservoir [7]. Most common complication is urinary tract infection reported in 60% patients and also presented in the present case. Intermittent and constant indwelling catheter may play an important role [8]. The bacterial strains growing in the novel reservoir change spontaneously and correlates poorly with increased antibody levels, thus indicating colonization rather than infection. However, asymptomatic bacteriuria must be treated in pregnancy, because of its association with preterm delivery [9]. Intestinal secretory loss and renal wasting result in electrolyte abnormalities such as hypokalemia, hypocalcemia, and rarely hypomagnesemia. Hypochloremic metabolic acidosis may appear, caused by reabsorption of ammonium chloride and secretion of bicarbonate [9]. Fortunately, none of these disturbances were observed in the present case. According to the literature, stenosis of anastomosis, recurrent infection, and urinary lithiasis (due to the presence of hyperchloremic metabolic acidosis, presence of foreign materials - sutures and staples and intestinal mucus) might impair renal function. Long-term annual monitoring of renal function is mandatory as it has been shown that glomerular filtration rate might decrease 15–25%, years after urinary diversion. Use of renal ultrasound and assessment of serum creatinine level is considered a screening method. In the present case, renal ultrasound was performed in 24th week of gestation, was within the normal range, and subsequent ultrasonographic evaluation was scheduled after the delivery. Patient was closely observed for potential problems associated with prescribed medications which are secreted in urine and, therefore, can be reabsorbed by the intestinal segments that are incorporated in the urinary tract. None of them occurred. Neurological deficits and megaloblastic macrocytic anemia, due to the possible long-term vitamin B12 malabsorption, were not present in the present case. One of the main reasons for poor quality of life after urinary diversion is diarrhea due to diminished bile salt and fat absorption. No stoool problem appeared.

As the authors have shown, regular bacteriological analysis of urine and rapid aggressive treatment at an early stage are indicated to reduce the incidence of premature labor and fetal morbidity. Renal function must be followed carefully with serum creatinine levels monitored monthly and if increased, the upper tract promptly evaluated by renal ultrasound. Management of labor needs to be assessed individually. Although concern about previous surgery results in most patients having an elective cesarean section, successful and safe vaginal delivery is possible.

Conclusion

Pregnancy and vaginal delivery are not contraindicated after ileocystoplasty, and in selected cases might be considered safe both for mother and baby. Good obstetric and urological outcomes can be achieved by close interdisciplinary monitoring of the pregnant patient and her fetus, and mutual decision-making.

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Pregnancy and delivery after vesico ileocystoplasty – a case report


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Posterior reversible encephalopathy syndrome in obstetric patients. Report of three cases with literature review

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Summary

Posterior reversible encephalopathy syndrome (PRES) is a serious clinico-neuroradiological maternal complication in pregnancy. Although it has various etiologies such as hypertensive encephalopathy, renal failure, autoimmune disorders, sepsis, multiple organ failure, and treatment with immunosuppressant or cytotoxic agents, pregnancy and postpartum complicated by hypertensive disorders more frequently lead to this condition. PRES is clinically characterized by headache, confusion, seizures, vomiting, and visual disturbances with radiographic vasogenic edema especially affecting symmetrical parietal and occipital lobes. The underlying pathophysiology is still a matter of debate. Prompt recognition and early intervention greatly improve the prognosis, so that obstetricians should be well aware of this rare entity. Timely imaging is of crucial importance especially in patients with an uncertain diagnosis for determining the appropriate treatment and preventing the possible development of neurologic deficits. In the present report, three cases of PRES are presented with clinical and radiological findings in pregnancies complicated by severe pre-eclampsia and eclampsia. The latest literature in the field is also carefully reviewed.

Key words: Encephalopathy; Eclampsia; Pre-eclampsia; Hypertension.

Introduction

Posterior reversible encephalopathy syndrome (PRES) is a transient clinical neuroradiological entity characterized by clinical signs and symptoms including hypertension, headaches, generalized seizure activity, altered mental status, and visual disturbances, as well as characteristic findings on brain magnetic resonance imaging (MRI) scan [1]. It was first described by Hinchey et al. [2] in 1996; since then, both its clinical spectrum and underlying pathophysiology remain poorly understood. Despite its rarity, PRES is most commonly reported in the literature in association with pregnancies complicated by pre-eclampsia and eclampsia. Furthermore, PRES is known to occur in a wide range of predisposing factors and causes, such as hypertension, solid organ and bone marrow transplantation, sepsis, autoimmune diseases, thrombotic thrombocytopenic purpura, renal failure and medication, and immunosuppressant and cytotoxic drugs [3-6].

Neuroradiological MRI findings usually include extensive symmetric bilateral hypertensity in T2 weighted images of parietal and occipital subcortical white matter and in the corresponding cortical regions in patients with PRES [7]. However, frontal lobes, basal ganglia, cerebellum, and brainstem may also be affected [6,8]. Advances in radiology and increasing expertise in the field provide the rising diagnosis of PRES.

Although the underlying pathophysiology is not fully understood, several responsible factors have been proposed such as rapidly developing hypertension leading to a breakdown in cerebral autoregulation and vasogenic edema, especially in the posterior head region or endothelial dysfunction, or vasospasm with subsequent ischemia [8-10].

The prognosis of PRES is usually benign with complete reversal of clinical symptoms and radiological signs in case of early, adequate, and effective treatment which comprises control of elevated blood pressure and withdrawal of potentially offending agents. Despite its name, PRES may lead to permanent imaging abnormalities and secondary complications such as status epilepticus, intracranial hemorrhage, ischemic infarction, which may cause residual neurological sequel with substantial morbidity, and mortality [8,11]. Therefore, medical awareness of PRES, which is a diagnostic challenge for obstetricians, is of crucial importance.

In the present report, three cases of PRES are presented with clinical and radiological findings in pregnancies complicated by severe pre-eclampsia in one case, and by eclampsia in two cases. Neuro-imaging findings were emphasized. Latest literature in the field in obstetric patients is also carefully reviewed.

Case Report

Case 1 was a 27-year-old primigravida with singleton gestation and uneventful antenatal period. She presented with acute onset headache and one episode of seizure at 37 weeks. On arrival blood pressure was 140/90 mmHg. As she developed another tonic-clonic seizure at the time of ultrasonographic examination, a provisional diagnosis of eclampsia was made and...
she was immediately shifted for an emergency cesarean section where she delivered a healthy baby. HELLP syndrome was diagnosed in postoperative period. She lost consciousness 25 hours after cesarean section associated with blood pressure values of 240/110 mmHg. She was referred to intensive care unit with tracheal intubation.

Case 2 was a 28-year-old primigravida who presented with amniotic fluid leakage at the 33rd gestational week. She was diagnosed with pre-eclampsia at the 31st week. As she complained of severe headaches and visual abnormalities and the blood pressure rose up to 180/100 on arrival, she was shifted for cesarean section. HELLP syndrome was diagnosed in postoperative period. She was referred to intensive care unit. About 30 hours after delivery the patient again complained of sudden visual abnormalities similar to those before the operation. No seizure activity was noted.

Case 3 was a 34-year-old woman at 31 weeks gestation. She presented with increased blood pressure and severe headaches. She stated that she did not come regularly for routine antenatal examinations and had a previous cesarean delivery. Her blood pressure on admission was 190/110 mmHg and urine laboratory evaluation showed 4+ proteinuria. As she developed severe headaches, visual abnormalities, and epigastric pain, emergency cesarean section was performed. Postoperatively she was taken to the intensive care unit. At postoperative first day she developed tonic-clonic seizure activity and HELLP syndrome.

All of the patients presented in the present report received appropriate antihypertensive treatment for blood pressure control as well as intravenous magnesium sulfate (MgSO4) treatment for seizure prophylaxis. In their neurologic evaluation no focally neurologic pathologic finding was examined. Ophthalmologic evaluation revealed residual perception of light, reactive pupils, and normal fundi in all cases. They were discharged from the hospital at 17th, 12th, and 14th postoperative days, respectively, with complete resolution of clinical and radiological features.

In all three patients MRI scan of the brain was performed. Axial T2 and fluid attenuated inversion recovery (FLAIR) MRI sequences demonstrated subcortical white matter hyperintensity lesions in occipital and parietal lobes in keeping with vasogenic edema. Additionally, case 2 also demonstrated white matter hyperintensity in T2 weighted FLAIR images in the pons. Diffusion weighted imaging (DWI) axial images demonstrated signal distortion secondary to edema but acute infarction was not suspected confirming PRES (Figure 1). Basal ganglia and capsula interna were normal.

Discussion

PRES is an acute rapidly evolving clinical condition characterized by clinical signs and symptoms including hypertension, headache, generalized seizure activity, altered mental status, and visual disturbances as well as characteristic findings on brain MRI scan [1]. Although the underlying pathophysiology of PRES is still a matter of debate, several theories have been suggested, the most widely accepted of which states that rapidly growing hypertension leads a breakdown in cerebral autoregulation, particularly in the posterior head region where there is a relative lack of sympathetic innervation. Hyperperfusion ensues with protein and fluid extravasation, producing focal vasogenic edema [8]. The second theory implicates endothelial dysfunction as it is defined in pre-eclampsia and eclampsia. A third theory proposes that vasospasm with subsequent ischemia may be responsible. It suggests that increases in the blood pressure lead to cerebral vasoconstriction and ischemia causing cytotoxic edema [8-10]. While the majority of cases resolve with treatment, PRES is not always reversible, not always limited to the posterior regions of the brain and not limited to the white matter. Irreversible neurologic deficits including delayed onset seizure disorder and even death have also been reported [9, 12].

Although literature is based mainly on case reports, few studies especially from neurology and radiology clinics are available. Fugate et al. [8] identified 120 cases of PRES in different clinical subgroups, of which seven were...
obstetric cases. They reported that the most commonly involved brain regions were parieto-occipital lobes (94%), followed by the frontal lobe, temporal lobe, and cerebellum. They also found cerebellar involvement to be more frequent in patients with a history of autoimmunity and patients with preeclampsia were more likely to have cortical involvement. The location and severity of vasogenic edema were mostly similar in different groups. Mueller-Mang et al. [4] also indicated no difference in distribution of lesions and extent of disease between patients with or without pre-eclampsia/eclampsia. Roth and Ferbert [6] conducted a study on 21 patients and found no difference with regard to symptoms, cerebral imaging and outcome, apart from a difference in age, the premedical history, whereas headaches occurred more frequently in the pregnant group. Similarly Liman et al. [3] mentioned that headaches were more frequent as initial symptom. However, in contrast, Liman et al. [3] found major clinicoradiological differences between obstetric and non-obstetric patients pointing toward a less severe course of disease in obstetric group. Their results showed altered mental state and affection of thalamus, midbrain, and pons were less frequent in the obstetric group. They also concluded that obstetric group had less severe edema, less cytotoxic edema, hemorrhage and contrast enhancement, while more frequent complete resolution of edema and less frequent residual structural lesions were seen on follow up imaging.

Although PRES is known to occur in a wide range of predisposing factors and causes, it is most commonly reported in the literature in association with pregnancies complicated by pre-eclampsia and eclampsia. Wagner et al. [13] suggested that neuroimaging showed characteristics of changes of PRES in all seven of 13 eclamptic cases in whom neuroimaging studies were available. More recently Brewer et al. [14] reviewed 47 eclamptic patients in order to investigate the concurrence of PRES with eclampsia. They indicated that PRES is a core component of the pathogenesis of eclampsia as 46 of 47 (97.9%) of eclamptic patients revealed PRES on neuroimaging studies. Their results also demonstrated that severe systolic hypertension was present in 47% of patients. It is possible that blood pressure alone is not the exclusive cause, and that the endothelial dysfunction which is a hallmark of pre-eclampsia, is also a contributing factor. Alternatively, pregnancy itself may decrease the threshold at which an elevation in the blood pressure may lead to cerebral hyperperfusion and brain edema [15].

The widespread use of MRI technology has made PRES familiar to many clinicians in the last decade. Specific MRI techniques, such as FLAIR and DWI sequences have improved the ability to detect subcortical/cortical lesions and helped to clarify the underlying pathophysiological mechanism of cerebrovascular involvement, which results important for an appropriate therapeutic decision. DWI sequences of the brain are considered to be the gold standard. Cerebral edema is seen as an increased T2 weighted signal which may be tricky to detect if the lesions are small and subcortical, as they may be difficult to distinguish from adjacent cerebrospinal fluid (CSF). FLAIR sequences suppress the CSF signal and make these abnormalities more conspicuous. DWI signals and apparent diffusion coefficient (ADC) map values may help the radiologist to elucidate a diagnosis. Unlike areas of arterial infarction, lesions suspicious for PRES tend to display no restriction of diffusion on DWI. This lack of restriction of diffusion has led to the belief that the edema seen with PRES is not caused by cell swelling “cytotoxic edema” which is thought to occur with ischemia, but must be caused by leakage of fluid from the vasculature “vasogenic edema” [16, 17].

Here three cases of PRES are presented with clinical and radiological findings in pregnancies complicated by severe pre-eclampsia in one case, and by eclampsia in two cases. Severe headache was the initial symptom in all cases as it is indicated in the aforementioned studies [3, 6]. PRES was diagnosed on the basis of the results of neuroimaging studies. Published reports in the literature suggest an important question whether it is necessary to perform imaging studies routinely in patients with a classical presentation of eclampsia or not. Although PRES is most recently proven to be the core component of the pathogenesis of eclampsia, [14] several conditions such as acute stroke, intracranial emboli or hemorrhage, systemic diseases that are associated with central nervous system may mimic eclampsia. Differential diagnosis among these conditions, which is difficult on the basis of clinical findings alone, may not only affect treatment modalities but also act on long term neurological outcomes. Aggressive blood pressure control is the main target in PRES whereas acute stroke permits mild to moderate hypertension. Eventually timely imaging is of crucial importance especially in patients with an uncertain diagnosis for determining the appropriate treatment and preventing the possible development of neurologic deficits.

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Psychosexual and social consequences in a woman with undiagnosed Mayer-Rokitansky-Küster-Hauser syndrome

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Summary

Purpose: To report a woman with devastating psychosexual and social consequences as a result of undiagnosed Mayer-Rokitansky-Küster-Hauser syndrome (MRKH) syndrome. Materials and Methods: An 18-year-old woman was referred after being divorced for “absent vagina and uterus”. On examination, secondary sexual characteristics were normal. Vaginal and rectal examinations revealed absent vagina and uterus. Investigations showed normal hormonal profile, 46 XX karyotype, and normal intravenous pyelography. Pelvic ultrasonography and magnetic resonance imaging (MRI) confirmed the absence of the uterus and presence bilateral ovaries. Results: A diagnosis of MRKH syndrome was made and she underwent successful modified laparoscopic Vecchietti operation for creation of a new vagina. Vaginal dilators were used after the surgery. Two years of follow up confirmed that vaginal length was about ten cm. Conclusion: The proper diagnosis, counseling, and prompt treatment of MRKH syndrome can prevent tragic consequences.

Key words: Intercourse; Mayer-Rokitansky-Küster-Hauser syndrome.

Introduction

Mayer-Rokitansky-Küster-Hauser syndrome (MRKH) is defined as the congenital absence of the vagina and of the uterus [1]. Anomalies of the urinary tract and the skeletal system are commonly associated with the syndrome. The exact incidence of the syndrome is unknown. It is estimated to be of 1:1,500 to 1:4,000 infants [2]. The etiology remains unclear. It has been considered to be a multifactorial anomaly, but there is some evidence to suggest a genetic defect that is transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity [3]. The condition usually goes unperceived until puberty. Then the clinical presentation is usually primary amenorrhea, with a normal ovarian function, normal female karyotype, and secondary sexual characteristics. Anomalies of the urinary tract and the skeletal system are commonly associated with the syndrome. In the present case, the objective was to highlight an atypical presentation of MRKH syndrome.

Case Report

An 18-years old woman was referred to King Abdulaziz University Hospital after being divorced for “absent vagina and uterus.” She was married for five months and sought medical advice for painful and difficult intercourse. She was told that there was congenital absence of the vagina and the uterus and therefore cannot have children. Consequently, she was divorced because of the sexual difficulties. She was seen four years ago for primary amenorrhea but was re-assured. On examination, her weight was 60 kg and height was 168 cm. Secondary sexual characteristics were normal. Vaginal and rectal examinations revealed absent vagina (Figure 1) and uterus. Investigations showed normal hormonal profile, 46 XX karyotype, and normal intravenous pyelography. Pelvic ultrasonography and magnetic resonance imaging (MRI) confirmed the absence of the uterus and presence bilateral ovaries. A diagnosis of MRKH syndrome was made and she underwent successful modified laparoscopic Vecchietti operation for creation of a new vagina [4]. Vaginal dilators were used after the surgery. Two years of follow up confirmed that vaginal length was about ten cm and that the vaginal width easily accommodated two fingers.

Discussion

MRKH syndrome is the second most frequent cause of primary amenorrhea. Treatment of MRKH syndrome is controversial. Numerous non-surgical and surgical approaches exist in the literature. The aim is to create a neovagina of adequate size and physiologic condition to permit normal sexual intercourse. The American College of Obstetricians and Gynecologists recommends non-surgical vaginal dilatation method as a first-line approach [5]. Edmonds et al. in 2012, reported achieving successful vaginal length (de-
fined as greater than six cm in length and maximum width throughout the vagina and especially at the apex) and sexual function in 232 (94.9%) women out of 245 women using vaginal dilatation [6]. The surgical approaches include many vaginal (McIndoe, Sheare’s, and modified Williams), laparoscopic (Vecchietti and Davydov), and open transabdominal procedures [7]. In the United States the most commonly used operation for the creation of a neo-vagina is the McIndoe, while in Europe the Vecchietti procedure is gaining popularity [8]. The advantage of the laparoscopic Vecchietti method is the creation of a longer neovagina (9.6 cm mean length) with a normal anatomy, histomorphology, and functionality in 47.5 minutes (mean operative time) and 4.8 days (mean traction days) [4]. Because vaginal malformations are rare and occur on a wide spectrum, it is frequently difficult to diagnose. This is illustrated in the present case. She was undiagnosed when she presented with primary amenorrhea. It is of paramount importance to understand the anatomy, preparedness, preference, and expectations of each individual case to offer optimal treatment. The aim is to maintain the woman’s self-integrity and minimize the consequences of the diagnosis of MRKH syndrome. Creation of a neovagina may help in having sustained marital relationship. Unfortunately, as a result of the lack of the proper diagnosis and treatment, the present case was subjected to the devastating psychosexual and social consequences. Timely creation of a functional neovagina that is satisfactory for sexual intercourse is an essential part of the management of MRKH syndrome to minimize short and life-long consequences.

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