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Tel. +1-514-4893242 – Fax +1-514-4854513 – e-mail: canlux@mgroup-online.com
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Editorial Office (M. Critelli):
Via Martiri della Libertà, 9, 35137 Padua (Italy)
Tel. +39-049-656521 – Fax +39-049-8752018 – e-mail: irog.canada@gmail.com

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A case of a sudden antenatal death by severe strangulation is reported.

ERRATA CORRIGE

Expression and significance of CD133 and ABCG2 in endometriosis

Errata:
page 775.
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Corrige:
This project was supported by the Zhenjiang Foundation of Science and Technology, China (Grant No. SH2009019) and the Natural Science Foundation of Jiangsu, China (Grant No. BK20140502).
Dear Editor,

Qiao et al. [1] introduced a novel method to avoid intrauterine balloon prolapse: they placed sutures at the lateral cervix lips (Qiao suture) and, thereby, made the cervix narrow, which prevented intrauterine balloon from prolapse to the vagina, achieving hemostasis for postpartum hemorrhage (PPH). However, my concern is that the balloon may still prolapse from this narrow cervix: at cesarean section for placenta previa, a balloon frequently slides out through “narrow” cervix. Previously, our team introduced the “holding the cervix” technique both to achieve hemostasis of PPH general [2] and to prevent balloon from sliding out [3, 4]. Here, we propose a concept of combined use of Qiao suture and the “holding the cervix” to prevent balloon prolapse. We also would like to describe our experience on the safety of the latter technique.

First, the addition of the “holding the cervix” to Qiao suture may more effectively prevent balloon prolapse than Qiao suture alone. In the “holding the cervix” technique, both anterior and posterior cervical lips are closed by sponge forceps (Figure 1a) [2]. The blood, having no exit to the vaginal side, accumulates within the uterus, tamponading the uterine bleeding surface, leading to hemostasis. Our team applied this “holding the cervix” technique to prevent the balloon prolapse. After placing the balloon within the uterus (or the lower segment), the cervix should be closed, which completely prevented balloon prolapse [3, 4]. This becomes, when indicated, our department protocol for PPH. Especially when the cervix is still wide even after placing Qiao suture: addition of the “holding the cervix” may lead to improved hemostasis (Figures 1b-d).

Second, we would like to describe our experience that the “holding the cervix” did not damage the cervix being held. Although we were the first to report the “holding the cervix”, Kawamura et al. [5] also reported its effectiveness for preventing balloon prolapse, as Qiao’s et al. quoted. Qiao et al. were concerned about its adverse events. The forceps have been usually removed after 12-24 hours and we
have experienced no cervical injuries, damages, or ischemic events: its safety is already time-tested. The uterine cervices appeared normal at postpartum one-month check-up.

I ask Qiao and colleagues to consider the addition of “holding the cervix” technique to Qiao suture, when the cervix is still wide even after placing Qiao suture. In doing so, the concealed intrauterine or extrauterine (intra-abdominal) bleeding should be closely monitored and its absence should be confirmed.

Acknowledgement

Our team previously described the “holding the cervix” technique elsewhere, which I cited appropriately.

References


Reply by Qiao et al.

Thank you for your interest in our method, Qiao suture. You also propose a concept. However, we believe that every method is effective, and has a different mechanism to arrest hemorrhage. So we propose there is no need to combine them.

Address reprint requests to:
S. MATSUBARA, M.D., Ph.D.
Department of Obstetrics and Gynecology
Jichi Medical University
3311-1 Yakushiji, Shimotsuke
Tochigi 329-0498 (Japan)
e-mail: matsushi@jichi.ac.jp
Pre-eclampsia and the vascular endothelial growth factor: a new aspect

A. Liberis, G. Stanulov, E. Chafouz Ali, A. Hassan, A. Pagalos, E.N. Kontomanolis
Department of Obstetrics & Gynecology, Democritus University of Thrace, Alexandroupolis (Greece)

Summary
Pre-eclampsia (PE) is a multi-system disorder of human gestation characterized by hypertension, proteinuria, and edema, which resolves with placental delivery. This disease affects 3-14% of all pregnancies worldwide and 5-8% in the USA. Furthermore PE remains one of the leading causes of maternal and neonatal mortality and morbidity worldwide. One of the most important goals in obstetrics is the early identification of the patient with an increased risk for PE. This paper unifies the essential and validated findings of past and current scientific investigation which encompass the relationship between PE and the vascular endothelial growth factor (VEGF). VEGF and its receptors have acquired great interest due to their vital role in neovascularization (vasculogenesis and angiogenesis) in a variety of physical and pathological processes such as the female reproductive cycle, PE, and tumorigenesis. VEGF is secreted in response to tissue hypoxia and endothelial cell damage. Alterations in the circulating levels of this factor may therefore identify those pregnancies with a high possibility of developing PE. This review will summarize the present authors’ current understanding of the role of circulating VEGF in the pathogenesis, clinical diagnosis, and prediction of PE.

Key words: Pre-eclampsia; Hypertension; Gestational hypertension; VEGF; Pregnancy; Cytotrophoblast.

Introduction
Pre-eclampsia (PE) is the hypertensive disorder of pregnancy, presented with two significant phenotypes, high blood pressure, and proteinuria after 20 weeks’ of gestation [1, 2]; in multiple gestation and molar pregnancy, PE can also manifest itself in the same clinical type before the 20th week. PE is divided into preclinical and clinical stages; the term comes from the Greek word “lighting”. It is the final stage prior to eclampsia, a state characterized by generalized grand-mal seizures [3-6]. The disease is so-named as the precursor to eclampsia, whereas the child-bearer experiences a new onset generalized grand mal seizure; that is the novel seizure manifestation. The preclinical part is characterized by abnormal placentation before the 20th week of gestation; the clinical part is marked by elevated blood pressure, proteinuria, inadequate cytotrophoblast invasion, and systemic endothelial dysfunction after the 20th week [7-10].

The diagnostic criteria include the following: systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg and proteinuria defined as urinary excretion of ≥0.3 grams in 24-hour collection with no evidence of urinary tract infection [11, 12]. The disease is characterized by visual disturbances, edema, seizures, headaches, epi-gastric pain or right upper quadrant pain, impaired hepatic function, hemolytic anemia, elevated liver enzymes, low platelet count (HELLP syndrome), and intrauterine growth restriction. In addition to elevated blood pressure and proteinuria levels, severe PE is based on the following criteria: oliguria (< 500 ml in a 24-hour sample), cerebral hemorrhage, visual disturbances, pulmonary edema, and liver rupture [11, 13, 14]. Eclampsia can be complicated by placental abruption, renal failure, subcapsular hepatic hematoma, preterm delivery, convulsions, cerebral hemorrhage, and even fetal or maternal demise [11, 13].

Placental tissue is necessary for the evolvement of PE. Removal of the placenta after delivery converts the maternal state back to normal; the syndrome may persist if placental tissue is retained. These observations have led investigators to consider that there is perhaps a circulating factor or factors produced by the placenta contributing to maternal disease.

Pregnancy is thought to be a cardiovascular stress test; it can be a tool of detecting women with an increased risk of developing vascular disease. Risk factors such as hypertension, diabetes, obesity, a previous history of PE and the presence of coronary artery disease (CAD) increase the risk for PE, leading to severe early-onset disease with greater...
frequency and also with adverse neonatal outcomes [7, 18]. Efficient placental function guarantees the normal exchange of nutrients between the fetus and maternal side [7, 15]. The under-perfused placental mass releases a variety of placental factors into the maternal circulation affecting vascular spurring, growth and permeability, and endothelial dysfunction [15-18]. Impaired trophoblastic invasion of the maternal placental area combined with the imbalance between the angiogenic factors are considered to be the initiation steps of endothelial dysfunction in PE, resulting in impaired uteroplacental blood flow and subsequent local placental hypoxia [8, 18, 19].

**VEGF-polymorphisms of VEGF**

VEGF is the essential molecule secreted by the cytотrophoblasts and guarantees the vasculature stabilization in the human organism. It is a heparin–binding, dimeric glycoprotein of 45,000 daltons member of the platelet derived growth factor (PDGF) family of mitogens with potent angiogenic properties secreted in response to tissue hypoxia and endothelial cell damage. It acts through transmembrane tyrosine kinase receptors on vascular endothelial cells. VEGF exists in four molecular types which have respectively 121, 165, 189, and 206 amino acids. VEGF is a cytokine molecule, essential for endothelial integrity and stabilization with vasoactive properties; it induces nitric oxide and prostacyclin synthesis by endothelial cells, endothelial cell proliferation, and migration. The two main VEGF receptors are the Flk-1 (VEGFR-2) and the Flt-1 (VEGFR-1). The interaction of VEGF and placental growth factor (PIGF) with their endogenous receptors is prevented by sFlt-1 which is located on monocytes. In PE pregnancies we anticipate the presence of oxidative stress, systemic inflammation, and fluctuations in the circulating levels of the angiogenic factor. [2].

The crucial role of physiological vasculogenesis and vascular permeability is assigned to VEGF. VEGF is known to be a useful marker of early vascular development. The location of the gene encoding VEGF is on chromosome 6 band p21 and comprises a 14 kb coding region with eight exons and seven introns. The chromosome’s VEGF family displays different biological activities, due to their different specificities for the known receptors. Its properties are exerted through the existence of the two high-affinity tyrosine kinase receptors, Flt-1 (also known as VEGFR-1) and kinase insert domain receptor (KDR in humans)/(Flk-1 in mice), (also known as VEGFR-2); a membrane and a soluble isoform compose both receptors [9, 11, 12, 20-22]. In several studies in both animals and humans, the effect of blockage on VEGF action has been suggested to be the milestone of the pathophysiology of PE. VEGF signaling is critical for the establishment and maintenance of the glomerular filtration barrier; in this concept, anti-VEGF therapy produces proteinuria. It is possible that decreased VEGF activity produces the renal features of PE. In a wide range of renal diseases, the deregulation of VEGF expression has been demonstrated within the glomerulus. Therefore, we would expect that VEGF polymorphisms might affect the occurrence of PE [17].

Clinical studies in women have shown that circulating total VEGF concentrations are significantly decreased in women with PE. VEGF is thought to be involved in the pathogenesis of PE rather than being an effect of the disease. Genetic polymorphisms on VEGF could affect the susceptibility to the development of PE or gestational hypertension (GH); this is seen in relevance to VEGF in normal pregnancy; the abnormalities in VEGF function are possibly associated with PE or GH. Family studies have shown that genetic factors might play a role in PE; however the exact inheritance pattern is still unknown. A few of them have been correlated with variation in VEGF protein production [17]. Therefore, PE is characterized by normal to high total VEGF levels (probably induced by placental hypoxia) but low free VEGF levels, owing to a vast excess of sFlt-1 which antagonizes the VEGF effects on the formation of placental vasculature and maternal endothelial cell function. A second trimester analysis of circulating VEGF appears to be a useful tool for the early identification of pregnant women who are at increased risk for developing severe, early onset PE; measuring VEGF levels in the umbilical vein and artery and investigating maternal and fetal VEGF polymorphisms is informative regarding the possible associations between VEGF and PE [20].

Taking into account the essential role of VEGF in pregnancy, polymorphisms of the VEGF gene were assumed to be important markers to determine the liability to PE. Based on genetics, this interaction delineates the association between genetic polymorphisms of VEGF and an increased risk of developing PE [14].

Genetic polymorphisms of the VEGF gene, linked to an inherited alteration of VEGF production, may contribute to the pathogenesis of PE. The carrier state of the VEGF<sup>165G</sup> allele, which is accompanied by high VEGF-producing capability, decreases the risk of severe PE. An inherited ability of VEGF secretion could be protective against PE in relation to earlier clinical experience that VEGF has a potential effect on placentaion [13].

In conclusion, extensive genetic studies should be launched to improve statistical accuracy, to explore the functional applicability of VEGF polymorphisms and their relationship with PE [14].

**The pathophysiology of PE**

Normal pregnancy is associated with an increased endothelium-induced immediate response to angiotensin II, epinephrine, and increased blood flow [2, 23]. Blood flow to the uterus increases approximately tenfold in gestation, half of which goes to the placental unit and the fetus. Studies in
various animal models have shown that reduction in uteroplacental blood flow can lead to a hypertensive state that closely resembles PE [2, 7, 24]. The ischemic and poorly perfused placenta is thought to be the basis of the underlying pathology of PE [24]. PE originates in the placenta starting with inadequate cytotrophoblast invasion into the uterine spiral arteries and ending in widespread maternal endothelial dysfunction. PE is divided in three stages: abnormal remodeling of the placental bed vascular, placental ischemia, and dysfunction of the endothelial cells’ layer. Pathological development of the placental vessels results from insufficient trophoblast invasion of maternal spiral arteries during the first days of gestation. Abnormalities in trophoblast invasion and generalized maternal endothelial dysfunction seen in PE may be triggered via release of placental factors. Pre-eclamptic women demonstrate significantly increased levels of fibronectin, the Von-Willebrand factor, decreased NO production, increased ratio of thromboxane/prostacyclin; compounds that comprise the serum markers of endothelial cell injury [19, 23]. The concentration of serum markers of endothelial cell injury and abnormal placental development are reflected in maternal circulation [2]. The hypoxic and poorly perfused placenta induces a systemic inflammatory response that results in an altered vascular function by trophoblasts; the trophoblasts replace the endothelial cells of the spiral arteries. As a result the placental vasculature converts from a large-caliber to a low-capacitance high blood flow vascular network [3, 24, 25]. There is restriction of blood flow into the intervillous space leading to placental hypoxia. Placental ischemia leads to production of soluble factors that can cause maternal endothelial dysfunction. Under hypoxic conditions, the endothelial and neoplastic cells express proteins known as endothelin, IL-1a, and VEGF; these proteins invade the surrounding tissues [17, 26]. Hypoxia also initiates the expression of PDGF mRNA and VEGF mRNA in tissue cultures indicating that oxygen is an important angiogenesis regulator [25-27].

Additional clinical studies have shown, the syncytiotrophoblast, the villous cytotrophoblast, and the feto-placental vasculature expressing the HIF-1a and HIF-2a factors; they are present in pre-eclamptic placentas of any gestational age. HIF-2a is strongly expressed in pre-eclamptic placental tissue and not down-regulated upon oxygenation [28].

Clinical studies revealed, that female subjects with signs of PE and those giving birth to small-for-gestational-age (SGA) newborns show elevated plasma levels of the soluble form of the VEGF-1 and the soluble form of the Endoglin (s-Eng) molecules; VEGF and PIGF are low in concentrations in the same subjects [16, 27, 28]. As a consequence of placental hypoxia, there is production of sFlt-1 in PE, which is related to the failure of the cytotrophoblasts invading into the spiral arterioles. Decreased oxygen tension of normal villous explants cause a two-fold-elevation in sFlt-1 secretion level. Before the occurrence of PE, PIGF serum level is markedly decreased.

The PE syndrome follows secondary to aberrant placentation and excess placental secretion of sFlt-1. The starting point of PE is the placental mass; the placenta undergoes the pathways of vasculogenesis and angiogenesis; angiogenesis is the process of neovascularization from pre-existing blood vessels, whereas vasculogenesis is the process of blood vessel generation originating from the angioblast precursor cell. During fetal development, the human placenta is characterized by increased activity of angiogenesis and vasculogenesis. [3, 13, 23]. Delivery of the placenta allows the hemodynamic parameter of blood pressure to return to a normal level. Severe PE is related to placental hypoperfusion and ischemia; placental infarcts, acute athrosis, fibrin deposition, vascular intimal thickening, and endothelial damage can be detected. Uterine artery Doppler abnormalities are suggestive of increased impedance to blood flow in the uterine circulation and failure of the physiologic transformation of the spiral arteries, diagnosed through histological examination of the placenta [5, 10, 19].

As it has already been noted, during the early first steps of normal placental growth, fetal extra-villous cytotrophoblasts invade the uterine spiral arteries of the decidual superficial layer and the myometrium. Maternal spiral arteries are being transformed from small high-resistance vessels to large diameter capacitance blood vessels, having the ability to provide adequate blood perfusion to support fetal growth; in PE, this procedure is incomplete [29].

The role of angiogenic and antiangiogenic factors in placental vascular development

Angiogenic factors are the main contributors to placental vascular development; moreover their receptors are of great significance for normal placenta and embryo development [24, 30]. Expression studies show that the growth of the human placenta is related to angiogenic growth factors and their receptors. Hybridization and immunohistochemical studies of VEGFRs on the human placenta demonstrate that the receptors are localized in the villous trophoblast and macrophages of both fetal and maternal origin [17].

PIGF and VEGFR-1 (Flt-1) are being expressed in the trophoblasts; expression of these proteins is altered in PE. The main regulator of angiogenesis is sFlt-1, which is produced by splicing of the VEGF mRNA. The binding process of the VEGF receptors (VEGFR-1 and VEGFR-2) is prevented by sFlt-1 which causes low circulating levels of free VEGF, and PIGF [2, 11, 12, 16, 27, 28, 30]. VEGF and PIGF are produced in excessive concentrations in the trophoblastic villi in gestation and inactivated by sFlt-1; cytotrophoblasts are prevented from spreading out and developing in utero by the sFlt-1 molecule. The titer of sFlt-1 remains low during early gestation and reaches a maximum in the late third trimester; a reason why this biomarker cannot be used efficiently in the diagnosis of PE [17]. There is enhancement of the sFlt-1 molecule during the late third
trimester in the maternal circulation which increases with Eng. This may lead to maternal endothelial dysfunction and the clinical syndrome of PE.

The pathogenesis of PE is the result of the imbalance of the angiogenic and anti-angiogenic factors; there is an increased expression of soluble fms-like tyrosine kinase-1 (sFlt-1) and a decreased PIGF and VEGF expression profile [9, 10, 12, 16, 27, 31]. In experiments with pregnant rats it has been observed that vessel injection of sFlt-1 causes proteinuria and hypertension [32]. The structural integrity of the glomerular ultrastructure and the fenestration of the glomerular endothelial cells is an important biological property for VEGF [33].

Eng is the co-receptor for the transforming growth factors - β1 and -β3 located on the endothelial cells; it increases vascular permeability and induces high blood pressure in rodents. It has been observed that there are increased levels of the Eng molecule two to three months before the onset of PE. The molecules, s-Eng and sFlt-1 might lead to endothelial dysfunction and the syndrome of PE [2, 30]. Furthermore, the addition of sFlt-1 in pregnant rats results in the classical pre-eclamptic triad of hypertension, proteinuria, and renal endotheliosis [34].

It has also been observed that sFlt-1 and Eng show potent anti-angiogenic properties [12]. In addition s-Eng binds and antagonizes TGF-b (TGFb-1 and TGFb-3); it is upregulated in PE and is highly expressed in syncytiotrophoblasts and the cytotrophoblasts. In pre-eclamptic women, the s-Eng molecule is elevated in the blood at eight to 12 weeks before the clinical outbreak of the disease [17, 23, 26, 30]. s-Eng is a co-receptor detected in endothelial cells and the syncytiotrophoblasts. The genetic mutations on the Eng molecule two to three months before the onset of PE. The molecules, s-Eng and sFlt-1 might lead to the clinical syndrome of PE [2, 30].

SFlt-1 increases its serum concentration in pre-eclamptic females five to ten weeks before the onset of the disease, with concurrent serum fall of the free VEGF and PIGF titers, which are expressed in both bovine maternal and fetal tissues. This expression in relation with their receptors, VEGFR-1 and VEGFR-2, increases through the period of maximal placenta development [11].

Conclusions and future directions

Invasion of the uterine wall by fetal extravillous trophoblastic cell is the triggering event in PE. The clinical features associated with PE can be initiated by placental factors which enter the maternal circulation and cause endothelial dysfunction resulting in hypertension and proteinuria. Ischemia or hypoxia of the placenta forms the basis of PE, resulting from defective progression of the spiral arteries’ remodeling and placental angiogenesis. In women with PE, studies have revealed that the total VEGF titer is significantly elevated. In pre-eclamptic subjects the biologically active free VEGF concentration was decreased and the sFlt-1 concentration was elevated. The specific mechanisms which lead to excess sFlt-1 production by the placenta, the role that sFlt-1 plays in normal development and PE, the relation between the sFlt-1, PIGF, and VEGF factors in PE are still unknown. PE is multifactorial with an onset, severity, and progression that can be significantly different among child-bearers. There is no definitive predictive test available to target women who will develop PE. New prospects have been introduced by recent advances in the understanding of PE and the identification of women who are liable to manifest the disease. The regulation of angiogenic gene products and their role in placental angiogenesis and systemic vascular health compose the new aspect of pre-eclamptic therapeutic and diagnostic options. The majority of women who develop PE demonstrate the whole range of the clinical manifestations of the PE syndrome. The fact that many of the proposed biomarkers for PE are increased in smaller concentrations in normal pregnancy makes the identification of accurate biomarkers for PE more difficult. If signs of abnormal placental and endothelial dysfunction could be detected prior to the onset of the clinical disease, this would represent an extremely attractive field for emerging therapeutic strategies. Soluble angiogenic markers used either alone or in a combination with other markers would strongly encourage research in the diagnosis and screening of PE. It is hoped that one or more of the various markers associated with PE will prove useful as a potential screening tool to identify those women destined to develop PE in pregnancy. Early diagnosis gives patients an opportunity for an early and effective remedy. The development of new drugs that would be effective in treating or preventing this devastating disease could begin with the identification of circulating angiostatic factors such as sFlt-1.

Acknowledgments

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References

Pre-eclampsia and the vascular endothelial growth factor: a new aspect


Address reprint requests to:
E.N. KONTOMANOLIS, M.D., Ph.D.
Demokratias Street, 74
Alexandroupolis 68100 (Greece)
e-mail: mek-2@otenet.gr
Summary
Purpose: To evaluate the efficacy of intralipid intravenous infusion in achieving a live pregnancy following IVF – embryo transfer in women of advanced reproductive age (40-42 years).

Materials and Methods: A matched control was performed. Women aged 40-42 with a previous history of miscarriage or who failed to conceive despite previous embryo transfer who entered an IVF program were offered intravenous intralipid therapy (four ml of 20% liposyn II in 100 ml normal saline over one hour) during the mid-follicular phase. Clinical pregnancy rates (eight weeks with viable gestation) and live delivered pregnancy rates were then determined and compared.

Results: The results were evaluated after ten matched cycles. There were no clinical pregnancies in those receiving intralipid vs. a 40% clinical and a 30% live delivered pregnancy rate in the untreated controls ($p = 0.087$, Fisher’s exact test). The study was terminated because of these preliminary data.

Conclusions: In the test tube, adding intralipid to natural killer cells can inhibit their cytolytic action. However, the use of intravenous intralipid to suppress natural killer cell activity does not seem to improve the chance of a live delivery in women aged 40-42 years with a previous history of miscarriage. In fact this therapy may actually be detrimental in this age group. Since efficacy of this therapy was not found in a group of advanced reproductive age, it is not clear why this should be effective for a younger population. A controlled study for the younger group is needed.

Key words: Recurrent miscarriage; In vitro fertilization; Intralipid; Advanced reproductive age.

Introduction
Based on preventing abortion in some murine experiments in 1994, Clark suggested that the treatment related to its lack of side effects and low cost could be considered for treatment in humans with recurrent miscarriage [1]. Clark stated that “for adequate statistical power, this would require a large multicenter, prognostically stratified randomized controlled trial (RCT) and suggested that this could be accomplished via the Recurrent Miscarriage Immunotherapy Trialists Group Network” [1]. Twenty years later no such RCT has been performed. Some studies especially by Roussev et al. have found that intralipid may suppress in vitro natural killer (NK) cell activity [2, 3].

Most small studies were performed by Coulam and Acacio who stated that intralipid treatment seems to be equally effective to the much more expensive intravenous immunoglobulin (IVIG) therapy [4]. However recently a multicenter cooperative RCT evaluates IVIG for recurrent miscarriage organized by Stephenson et al. questioned the efficacy of IVIG [5].

The objective of this present study was to evaluate whether infusion of IVIG to a group of women of more advanced reproductive age (40-42 years) with a history of failure to conceive with embryo transfer or miscarriage.

J.H. Check1,2, D.L. Check2
1 Cooper Medical School of Rowan University, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ; 2 Cooper Institute for Reproductive Hormonal Disorders, P.C. Marlton, NJ (USA)
**Materials and Methods**

A matched control was performed. Women aged 40-42 years with a previous history of miscarriage or who failed to conceive despite previous embryo transfer who entered an IVF program were offered intravenous intralipid therapy (four ml of 20% liposyn II in 100 ml normal saline over one hour) during the mid-follicular phase. Clinical pregnancy rates (eight weeks with viable gestation) and live delivered pregnancy rates were then determined and compared.

**Results**

The results were evaluated after ten matched cycles. There were no clinical pregnancies in those receiving intralipid vs. a 40% clinical and a 30% live delivered pregnancy rate in the untreated controls ($p = 0.087$, Fisher’s exact test). The study was terminated because of these preliminary data.

**Discussion**

Since efficacy of this therapy was not found in a group of advanced reproductive age it is not clear why this should be effective for a younger population. A controlled study for the younger group is needed. Perhaps such a study could be limited to only those with miscarriage rather than also concluding failure to conceive despite embryo transfer.

The title of an article written by Shreeve and Sadek nicely summarizes the present knowledge of intralipid therapy “Intralipid therapy for recurrent implantation failure: new hope or false dawn” [6]. Seventeen years after the 1994 article by Clark [1], these authors state “we have concluded that appropriately controlled, large-scale confirmatory studies are necessary to prove the efficacy of intralipid before it can be recommended for routine use” [6].

**References**


Address reprint requests to:
J.H. CHECK, M.D., PH.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com
Introduction

Ovarian teratomas are a group of tumors made up of mature and/or immature tissue derived from embryonic germinal cells following their first meiotic division [1]. They include tumors of different histologic types such as:

1. Mature teratoma or dermoid cysts, the most common, always have tissues from at least two of the three embryonic layers and show the following:
   a) Tissue from the ectodermic layer: skin, brain, neural tissue (always);
   b) Tissue from the mesodermal layer: muscle, fat, cartilage, bone (present in over 90%), and teeth (present in 31%);
   c) Tissue from the endodermic layer: gastrointestinal, bronchial, or thyroid tissue, and adipose tissue (present in 67% to 75% of mature teratomas).

2. Immature teratomas, which are very rare (less than 1%) and with high potential for malignancy.

3. Monodermic teratomas, where a single type of tissue predominates (struma ovarii with thyroid tissue, neuroectodermic tissue in carcinoid tumors, or teratomas of neural tissue, for example).

Although dermoid tumors can be easily diagnosed with ultrasound (US), they at times present image variations that make diagnostic accuracy difficult. The authors report that in their experience, diagnostic uncertainty can be avoided with the use of HDLive US.

Materials and Methods

In a 12-month period (January - December 2013), the authors used 2D/3D/4D vaginal US as well as HDLive US for the evaluation of 31 women with tumors tentatively diagnosed with benign ovarian cystic teratomas. These were consecutive women in which an ovarian teratoma was suspected. All women referred were re-examined with a high-resolution ultrasound machine. The comparison between 2D (orthogonal planes) and HDLive are shown in Figures 1, 2, 4, 6, and 7.

In 27 of these patients (87.1%), the authors confirmed the diagnosis of ovarian cystic teratoma with HDLive US while in the four other patients, other tumors were diagnosed: two endometriomas, one solid-cystic benign tumor, and one ovarian carcinoma, FIGO Stage 1A, all of which were confirmed following surgery.

The ultrasound images were classified based on their cystic, mixed, or solid nature (Table 1). The authors established the following types:
1. Cystic most frequent unilocular tumor, with or without a Rokitansky nodule and with or without sonic attenuation.

2. Solid homogeneous and diffuse mass with/without an echogenic area that showed attenuation.

3. Diffuse solid or solid-cystic mass with multiple very fine echogenic bands produced by hair, or other refringent tissues (bone, teeth, or cartilaginous nodules), known as dermoid nodules.

The authors used in addition certain images that are occasionally seen and which are described in the literature as specific of these tumours [2, 3]:

- a) The iceberg sign, refringent surface small masses with profound sonic attenuation.
- b) The presence of double levels: liquid/liquid, fat/liquid [4].
- c) Trabecular images: multiple thin echogenic dots and fine bands caused by hairs, bone, teeth, or other ectodermic structures. Also dermoid mesh was included [4].
- d) Dermoid plugs: a wide echogenic area with multiple linear echoes and white points [5].
- e) Fat balls floating freely in a great cystic mass.
- f) Partial cystic-partial solid tumors, with non-homogeneous inner tissues, also poorly delimited, that we have called non-homogeneous dermoid tumors.

All diagnoses except two were confirmed shortly after ultrasound diagnosis by anatomic pathology following surgery. Tumors seen in two pregnant women were confirmed belatedly by pathology following surgery after the period of lactation.

This study received the approval from the ethical committee of the Hospital Clinico Universitario of Valencia (Spain). Informed consent from all patients participating in this study was obtained for US exam and for the surgery.

**Results**

**Patients age**

Because of the embryonic nature of dermoid tumors, the authors have observed them at all ages. Six patients (22.2%) were younger than 25 years of age. Their tumors were diagnosed when they consulted for family planning, infertility, or pregnancy.

**Gravity and parity**

These are not significant factors, but in any case, 18 patients (66.7%) were nulliparous women.

**Tumor size and bilaterality**

Only four tumors (14.8%) were larger than ten cm in diameter. This allowed preservation of much ovarian tissue. Two women (7.4%) had bilateral dermoid cysts.

---

### Table 1. — Summary of patients characteristics such as age, gravity, and clinical diagnosis, as well as dominant ultrasound image of the tumor.

<table>
<thead>
<tr>
<th>Age</th>
<th>Gravida/Para</th>
<th>Size (cm)</th>
<th>Symptomatology</th>
<th>US image</th>
<th>US dominant image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>G3/P2</td>
<td>4.2 x 4.3</td>
<td>None, pregnancy</td>
<td>Solid with attenuation + bilateral</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>G1/P1</td>
<td>6.5 x 6.4</td>
<td>None, infertility</td>
<td>Fat balls</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>G0/P0</td>
<td>5.1 x 5.2</td>
<td>None, infertility</td>
<td>Solid with attenuation</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>G1/P0</td>
<td>4.4 x 3.7</td>
<td>None, pregnancy</td>
<td>Cystic, fluid-serous level, Rokitansky</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>G2/P2</td>
<td>2.35 x 1.40</td>
<td>None, menopause</td>
<td>Solid with attenuation and calcium</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>G1/P2</td>
<td>2.84</td>
<td>None, menopause</td>
<td>Cystic, mesh, and teeth</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>G0/P0</td>
<td>5.57 x 4.34</td>
<td>None, infertility</td>
<td>Fluid-fluid, Rokitansky, hairs</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>G1/P1</td>
<td>3.5 x 4</td>
<td>None</td>
<td>Solid with attenuation and calcium</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>G0/P0</td>
<td>2.15 x 1.79</td>
<td>None, infertility</td>
<td>Pure cystic with Rokitansky</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>G0/P0</td>
<td>Bilateral 8.4/10.15 - 6.8/5.8</td>
<td>Tumor</td>
<td>Solid – cystic with Rokitansky</td>
</tr>
<tr>
<td>11</td>
<td>41</td>
<td>G1/P0</td>
<td>50 x 42</td>
<td>None, post abortion</td>
<td>Solid with small cystic area</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>G0/P0</td>
<td>10.9 x 11.63</td>
<td>None</td>
<td>Solid with attenuation</td>
</tr>
<tr>
<td>13</td>
<td>52</td>
<td>G0/P0</td>
<td>2.3 x 1.6</td>
<td>None</td>
<td>Cystic with Rokitansky</td>
</tr>
<tr>
<td>14</td>
<td>47</td>
<td>G0/pP0</td>
<td>4 x 3 cm</td>
<td>None, menopause</td>
<td>Solid with cystic area</td>
</tr>
<tr>
<td>15</td>
<td>43</td>
<td>G2/P2</td>
<td>40 x 47</td>
<td>bleeding</td>
<td>Solid with a cyst</td>
</tr>
<tr>
<td>16</td>
<td>39</td>
<td>G3/P0/A1/C2</td>
<td>52 x 42</td>
<td>None, menopause</td>
<td>Solid with cysts</td>
</tr>
<tr>
<td>17</td>
<td>39</td>
<td>G1/P1</td>
<td>12 x 8</td>
<td>Pelvic pain</td>
<td>Solid dense with attenuation</td>
</tr>
<tr>
<td>18</td>
<td>37</td>
<td>G0/P0</td>
<td>3.7 x 3.5</td>
<td>Bleeding and pain</td>
<td>Fluid-fluid / fat-fluid level</td>
</tr>
<tr>
<td>19</td>
<td>39</td>
<td>G0/P0</td>
<td>4 x 4</td>
<td>None</td>
<td>Solid dense, hair</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>G0/P0</td>
<td>3 x 4</td>
<td>None</td>
<td>Solid dense, hair</td>
</tr>
<tr>
<td>21</td>
<td>51</td>
<td>G1/P0/Ao/C1</td>
<td>3 x 3</td>
<td>None</td>
<td>Cystic with Rokitansky</td>
</tr>
<tr>
<td>22</td>
<td>18</td>
<td>G0/P0</td>
<td>14 x 10 x 10</td>
<td>None</td>
<td>Cystic with Rokitansky</td>
</tr>
<tr>
<td>23</td>
<td>37</td>
<td>G0/P0</td>
<td>3.5 x 3.5</td>
<td>None</td>
<td>Solid with attenuation</td>
</tr>
<tr>
<td>24</td>
<td>35</td>
<td>G1/p1</td>
<td>3 x 4</td>
<td>Pain</td>
<td>Cystic with Rokitansky</td>
</tr>
<tr>
<td>25</td>
<td>31</td>
<td>G0/P0</td>
<td>2.2 x 2.6</td>
<td>None</td>
<td>Non homogeneous</td>
</tr>
<tr>
<td>26</td>
<td>80</td>
<td>G3/P3</td>
<td>8 x 8.7</td>
<td>None</td>
<td>Non homogeneous</td>
</tr>
<tr>
<td>27</td>
<td>39</td>
<td>G2/P0/C1</td>
<td>3.5 x 2.8</td>
<td>None, pregnant</td>
<td>Non homogeneous</td>
</tr>
</tbody>
</table>
Symptomatology
Because of early diagnosis, their size, and the propensity to grow without involvement of neighboring intra-abdominal organs, dermoid tumors are generally asymptomatic. Only two (7.4%) of the largest tumors (>ten cm in diameter) the authors observed were associated with symptoms (hypogastric pain and menorrhagia), and in these cases the symptoms could be attributed to other concomitant gynecologic pathology (uterine myomata).

Ultrasound appearance with HDLive
Dermoids present a wide spectrum of images depending on the predominant tissue type [3, 5]. In the vast majority of cases there are dense echogenic structures that correspond to complex masses of fatty tissue, sebum, hair, epithelial remnants, along with cartilage or bone.

Dermoids can also present as hyper-echogenic masses with posterior acoustic shadowing. These masses may also reflect focal or diffuse echoes like fine splinters or present double fluid-fluid or fat-fluid layers [2, 3, 5-7]. The present authors have observed a few images that were not pathognomonic of dermoid cysts such as:
1. Cystic, usually unilocular lesions with a tubercle or dense echogenic nodule, known as Rokitansky nodule, which may show acoustic attenuation due to the presence of hair, cartilage, or bone. This nodule is also known as a...
dermoid plug and represents a protuberance of the internal wall of the tumor where hair and other solid elements originate (Figures 1A and 1B).

2. Diffuse echogenic mass, usually with a small zone of sonic attenuation (not always present), that is basically produced by a large accumulation of sebaceous material (Figure 2).

3. Diffuse echogenic mass with numerous very fine and elongated bands of greater echogenicity caused by hair [8] (Figure 3).

The present authors consider the following ultrasonographic signs to be specific and pathognomonic of dermoid tumors:

1. Pure cystic lesions with bones, teeth, or cartilaginous nodules (generally called bones or dermoid plugs [9]. In these cases the papillae appear as protuberances with an acoustic shadow projecting into the cyst cavity. Hair, teeth, and bones typically arise from these nodules. (Figures 1 and later Figure 5).

2. The iceberg sign. It is very similar to the Asound attenuation phenomenon previously described. It is a mass with an amorphous, poorly defined echogenic focus in the near field that causes a posterior shadow and thus obscures the posterior portion of the lesion along with any structures behind it. The echogenic focus appears as a solid mass, but it is actually a cyst that contains a mixture of fatty liquid (i.e., sebum), matted hair, and cellular debris. The multiple tissue interfaces are responsible for the characteristic acoustic shadow. The fat mixed with the hair strands is echogenic and often attenuates the ultrasound beam. The reflective echo pattern is caused by the multiple tissue interfaces of the hair and sebum within the cystic mass. The acoustic shadow may totally obscure the back of the mass, hence, the term used for describing this image, Atip of the iceberg sign [10].

3. The fluid-fluid/fat-fluid levels. This type of layering within a cystic ovarian tumor strongly suggests a cystic
teratoma and is therefore considered pathognomonic of this tumor [11]. Pure sebum inside the cyst may be hypoechoic or anechoic. Fluid-fluid levels or fat-fluid interface result from sebum floating above an aqueous surface, which appears more echogenic than the sebum layer. There is a constant horizontal fluid level inside the mass that can change with the patient’s position (Figure 4).

4. The dermoid mesh [4]. A dermoid mesh with hyperechogenic calcifications indicates the presence of bone, teeth, or other ectodermal structure. These images are usually seen in a predominantly cystic medium. Hyperechoic solid mural components and hair-fluid levels represent multiple echogenic linear interfaces floating inside a cyst. All these interfaces represent hair fibers (Figure 4).

5. Dermoid plugs. These are in a vast hyperechogenic area with multiple bright linear echoes and spots [12]. (Figure 5.)

6. Multiple mobile spherical structures (fat balls) of slightly increased echogenicity floating free in a large cystic mass is one of the rarest patterns observed in dermoid tumors (Figure 6). In all cases some degree of mobility was observed with the application of abdominal pressure. In rare cases, a single ball that measures between four and seven cm can be observed [2, 13-26].

7. The non-homogeneous group. These are poorly defined cystic tumors with solid areas, always homogeneous, although poorly delimited, can be seen in their interior. These solid internal areas represent sebum. The combined use of transvaginal 2D and HDLive is required for diagnosis since both allow us to show clearly that the solid content consists of sebum and fat. Although the present authors have labeled it non-homogeneous, the nature of the solid areas, with or without whitish spikes, is pathognomonic.
The images that the authors saw most frequently were Rokitansky nodule in a cyst in six cases (cases 4, 9, 13, 21, 22, and 24), five cases (number 1 was bilateral) of diffuse solid mass with an area of attenuation (cases 1, 3, 8, and 12), four cases of diffuse solid mass with echogenic bands (cases 11, 14, 15, and 16), three cases of dermoid mesh (cases 6, 19, and 20), and three non-homogeneous cases (25, 26, and 27).

The present authors have seen isolated cases of pure unilocular or multilocular tumors (case 10, which was bilateral), of solid-cystic mass with calcification (teeth, bone, or cartilage)(case 5). Case 18 was a cyst with double liquid-liquid or fat-liquid levels and case 2 was a tumor with multiple balls.

Cases with dermoid plug and with the iceberg sign are very rare. The authors did not observe any with these findings among their cases. In five of the present cases there was a combination of images. In case 4 the authors saw a cystic area with a nodule of Rokitansky along with fluid-fluid/fat-fluid levels. Case 5 combined solid cystic area with calcium and acoustic attenuation. In case 6 there was a combination of double levels, solid-cystic mass with attenuation, and dermoid mesh. Case 7 had a combination of fluid-fluid/fat-fluid levels, a Rokitansky nodule, and a mesh. Case 8 combined a solid-cystic mass with a Rokitansky nodule and calcium.

Since several of the ultrasound images described are very similar (i.e., iceberg sign y echogenic mass with attenuation, or dermoid mesh with dermoid plug), if we catalogue them together, we can conclude that ultrasound images pathognomonic of dermoid are:
Figure 5. — Multiple small echogenic linear interfaces (mesh) are seen in the upper images. In the lower images two Aplugs can be seen inside the cyst. The small linear dots are hair and fibroid tissue.

Figure 6. — Fat balls seen in 2D shell mode and HDLive with and without maximal luminescence.
1. Cystic or solid cystic lesions with a Rokitansky nodule, with bone, teeth or cartilage (six cases, 22.2%)
2. The solid mass with or without attenuation that corresponds with pure sebum (five cases, 18.5%)
3. A diffuse mass with fine bands that correspond with hair inside sebum (four cases, 12.9%) and that may form meshes or plugs corresponding with a mixture of fat, sebum, and hair (three cases, 11.5%).

The sum of these last 12 cases (46.1%) of solid material add up to the most frequent US images observed in dermoid tumors. The authors therefore have available pathognomonic ultrasound images that enable them to accurately diagnose ovarian dermoid tumors in almost 100% of cases.

Discussion

It is evident that using HDLive improves the quality of images when compared with those obtained by imaging identical cases with 3D/4D [27]. Several publications regarding imaging in normal and pathological pregnancies, in folliculogenesis, and in gynecology agree on this point [27]. The present is the first report about the degree of improvement in diagnostic accuracy that can be achieved by using HDLive for the evaluation of a specific type of tumor, ovarian dermoid, which may reveal with 2D several complex images that compromise diagnostic accuracy. Of the 31 cases referred with a diagnosis of dermoid diagnosed by 2D transvaginal sonography, the authors agreed with the diagnosis in 27 cases (87%) with the use of HDLive, subsequently confirmed by pathologic evaluation of surgical specimens.

Certain US images like Rokitansky papilla, fluid-fluid/fat-fluid layers, and diffuse echogenic masses with or without sonic attenuation are evidently pathognomonic of benign teratomas. Because of the extreme rarity of other equally pathognomonic signs such as balls of fat, generate even greater interest when observed [14, 18, 28, 29]. The present authors show the first 3D/4D and HDLive images reported in the literature of these Aballs of fat.

The pathogenesis of dermoid ball formation is unknown. Several theories have been postulated:

a) Predominance of large secretory and absorptive rather than exfoliative surfaces lining the cysts would favor absorption of most of the contents into the general circulation, leaving the remaining material to solidify and mould into balls.

b) Each globule is formed by the aggregation of sebaceous matter around a tiny focus of debris, squamous cells, or a fine hair shaft while moving around in the cystic cavity.

c) The spherules are modeled as discrete masses rather than as amorphous masses because of the difference in physical and thermal properties of the material being deposited around each nidus. Floating balls require space to be remodeled.

d) This type of growth could be related to an unusual pattern of estrogen and progesterone receptor expression in the cystic teratoma [15].

No one has yet defined a method to test these theories [16, 17, 18]. These Aballs are described in classical German, English, and French literature and have been ascribed the interesting names that the present authors list in their original language [14]: Boules de graisse, Butterkugeln, Caviar-like bodies, Dermokugeln, epithelial balls, Erbsenartige Körper, fatty concretions, Fettkugeln, floating balls, inclusions, lipid globules, pill-like bodies, rounded balls, sebum balls, solid concretions, and spherules.

The first descriptions were microscopic [19] to be followed sometime later by radiologic descriptions using CT and MRI, and lastly, ultrasound description [8, 12, 14], report of three cases [15-18, 20-24], followed by five cases [25] and other reports [26]. Labeled as fat balls the microscopic findings were desquamative keratin, fibrin, hemosiderin, and sebaceous debris with skin squamous cells, fine hair shafts, and only a small amount of fat component. Some spherules had a two- to three-mm thick outer sebaceous shell [14].

As a differential diagnosis the authors can only postulate the possibility of lesser pelvis echinococcal infection, an exceptional event that takes place with daughter vesicles [18, 23, 26]. Dermoids may show hyperechogenic globules floating in a hypoechoic liquid. The echinococcal cysts or vesicles are hypoechogenic [30, 31].

References

HDLive ultrasound images of ovarian dermoid cysts: diagnostic accuracy


Address reprint requests to:
F. BONILLA-MUSOLES, M.D.
University of Valencia School of Medicine
Navarro Reverter 11
46004 Valencia (Spain)
e-mail: profesorbonillamusoles@hotmail.com
The effects of melatonin on endometriotic lesions induced by implanting human endometriotic cells in the first SCID-mouse endometriosis-model developed in Turkey

N. Yesildaglar1, G. Yildirim1, O.K. Yildirim1, R. Attar1, F. Ozkan2, H. Akkaya3, B. Yilmaz3

1 Department of Obstetrics and Gynecology, Yeditepe University, Istanbul; 2 Department of Pathology, Yeditepe University, Istanbul
3 Experimental Research Center of Yeditepe University (YUDETAM), Istanbul (Turkey)

Summary
Objective: To evaluate the effects of melatonin on endometriotic lesions induced by implanting human endometriotic cells in SCID mice. Materials and Methods: Prospective, randomized, controlled, experimental study. Experimental Research Center of Yeditepe University (YUDETAM). Thirty female, non-pregnant, nulligravid severe combined immunodeficient (SCID) mice. Endometriotic cells collected from patients with endometriosis were implanted subcutaneously in 30 SCID mice. These mice were randomized into two study groups: in the first group, mice were administered melatonin (20 mg/kg/day) following induction of endometriosis for four weeks; in the second group, nothing was administered. All the mice were given a high dose of exogenous estradiol (50 µg/kg/d, twice weekly). Four weeks after inoculation, necropsies were performed and endometriotic lesions were collected. All the lesions were evaluated histopathologically and the levels of SOD and MDA were assessed in the lesions. Results: Successful implantation was observed in the 28 mice that survived. Mean MDA level was 5.0±1.7 and 8.8±2.6 in the melatonin and control groups, respectively (p = 0.01); mean SOD level was 1.1 ± 0.1 and 1.0 ± 0.1 in the melatonin and control groups, respectively (p = 0.49). Mean histopathological score was lower in the melatonin group (p = 0.04). Conclusions: Melatonin was effective in the treatment of experimental endometriosis induced in SCID mice.

Key words: Experimental endometriosis; SCID mice; Melatonin.

Introduction
Endometriosis is an enigmatic disease characterized by the presence and growth of endometrial-like tissue outside the uterine cavity. Endometriosis is a major clinical problem with no cure. Current management of endometriosis is based on pain management, reduction in the volume of endometriotic lesions, and prevention of recurrence. Pain management options include non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, suppression of ovarian function with hormonal drugs (oral contraceptives, danazol, gestrinone, agonists, dienogest), and surgery (laparoscopic ablation or excision of endometriotic lesions) [1]. If infertility is the problem, then specific treatments like ovulation induction and insemination or in vitro fertilization might be the treatment option. Yet, there is inconclusive evidence to show whether all these modalities cure the disease. These agents also produce varying levels of side effects that in many cases prevent long-term use and can lead to poor compliance [2]. Thus, new pharmaceutical agents are required in the treatment of endometriosis that are non-hormonal and with few and acceptable side effects. In that sense, melatonin is a documented powerful free radical scavenger and a broad-spectrum antioxidant [3]. It has been shown by the present group that melatonin causes regression and atrophy of endometriotic lesions in rats [4]. Severe combined immunodeficient (SCID) mice are perfect experimental animal models for endometriosis research [5-9], since human endometriotic cells collected from endometriotic lesions can be implanted in SCID mice and endometriotic lesions identical to the lesions seen in women can be induced experimentally.

The objective of this project, which was the first research project carried out using SCID-mouse-endometriosis-model in Turkey, was to evaluate the efficacy of melatonin on endometriotic lesions induced by implanting human endometriotic cells subcutaneously in SCID mice.

Materials and Methods
In this project, 30 female, non-pregnant, nulligravid mature SCID mice weighing 21-25 grams were included and the experiments were conducted in the Experimental Research Center of Yeditepe University (YUDETAM). The mice were kept in micro-isolator cages and housed in a separate barrier facility in a well-controlled pathogen-free environment with monitored ambient temperature and regulated cycles of light and darkness. The mice were fed ad libitum with sterile laboratory chow and water. The
The study was approved by the Experimental Animals Ethics Committee of Yeditepe University. All experiments were performed in compliance with international guidelines on the ethical use of animals.

The use of human tissue for this study was approved by the Institutional Review Board of Yeditepe University. Human endometriotic cells were collected from human endometriotic lesions by laparoscopy or laparotomy for endometriosis surgery. The ages of the patients ranged from 21 to 46 years, with a mean of 34 years. None of the patients had received hormonal therapy or used NSAIDs continuously in the three months before they underwent surgery for endometriosis.

Small tissue samples collected from endometriotic lesions were dispersed in one ml of PBS and then they were implanted subcutaneously into the backs of SCID mice using a one-ml insulin syringe. In total 15 implantation sessions were performed and in each session human endometriotic cells were implanted in two SCID mice using an 18-gauge needle. Estrogen therapy was initiated at the time of injection and administered twice a week (50 µg/kg/d, s.c.); two weeks later the mice were randomized into the control group, in which no medication was administered, or in the treatment group, in which all the mice were administered 20 mg/kg/d melatonin s.c., commencing from the end of the second week following the implantation of human endometriotic cells (Figure 1).

Two weeks later all mice were euthanized under anesthesia and the endometriotic lesions were collected. The biopsies were fixed in 10% neutral buffered formaldehyde solution. All pieces were embedded in paraffin after routine dehydration and five-µm-thick sections were made with a microtome. The samples were stained with hematoxylin-eosin (HE). The slides were examined under a light microscope. The pathologist (F.O.) who assessed the samples was blinded to the treatment groups.

Morphological identification of the endometrial glands and stromas was the cornerstone of the microscopic examination. The persistence of epithelial cells in the implants was scored semiquantitatively as follows: 3 = well preserved epithelial layers; 2 = moderately preserved epithelium with leukocyte infiltration; 1 = poorly preserved epithelium (occasional epithelial cells only); 0 = epithelial line [10].

SOD activity was determined by the NWLSS NWK-SODO2 superoxide dismutase activity assay for mice. Activity was expressed as units per milliliter (U/ml). MDA levels were estimated by the NWLSS NWK-MDA01 assay for mice. Activities were expressed as µM.

The statistical analysis was performed using Student’s t-test. The data were expressed as mean ± SD and p < 0.05 was accepted as significant.

**Results**

A veterinarian monitored the animals’ health on a daily basis. No signs of significant local inflammation at either the operation or injection sites and no systemic reactions or infection were observed in any of the SCID mice included in the study. No significant difference was observed in the weights of the SCID mice. From a total of 30 mice in which human endometriotic tissue cells were implanted, 28 (93.3%) survived the procedure. Two mice died during the study right after estradiol injections.

Successful implantation was observed in 28 mice out of 28 (100%). On gross examination, the implants consisted of well-circumscribed nodules firmly attached to underlying tissue (Figure 2). The mean number of implants was 14 and 14 in the control and melatonin groups, respectively. Mi-
Croscopically, HE staining of implant sections demonstrated the presence of endometrial acinar glands in a mixed background of stromal and inflammatory cells. Mean MDA level was significantly lower in the melatonin group (5.0 ± 1.7 in the melatonin group vs. 8.8 ± 2.6 in the control group; \( p = 0.01 \)) (Figure 3). Mean SOD level was higher in the melatonin group (1.1 ± 0.1 in the melatonin group vs. 1.0 ± 0.1 in the control group; \( p = 0.49 \)) (Figure 4) but not significantly so, probably due to the number of SCID mice included in the study.

Volume analysis of the lesions was not performed, since the number of endometriotic cells implanted could not be standardized; however, in the examinations during necropsies, the lesions in the control group were found to be larger and more vascularized to some extent. Histopathological examination of the lesions was performed by an experienced pathologist who was blind to the specimens. Mean histopathological score of the lesions in the melatonin-treated group was significantly lower than the mean score in the control group (\( p = 0.04 \)). Mean uterine weights were 0.09 and 0.08 grams in the melatonin and control groups, respectively (\( p = NS \)).

Discussion

To the best of the present authors’ knowledge, this is the first study carried out to evaluate the effect of melatonin on the endometriotic lesions induced by human endometriotic cells in SCID mice. This is also the first SCID-mouse endometriosis-model developed in Turkey.

As early as the 1940s, Sampson proposed the theory of retrograde menstruation and implantation of endometrial fragments as the origin of endometriosis in women; yet since then only limited progress has been made to define the mechanisms associated with the etiology and pathophysiology of endometriosis. Since retrograde menstruation occurs in nearly all women of reproductive age [11], impairment of some additional factors is needed for the establishment and maintenance of endometriotic lesions. There are many other theories explaining the pathophysiological mechanisms of endometriosis, such as estrogen dependency, the role of a pro-inflammatory environment, and the effects of free radicals. However, at the time of clinical symptoms, most women already have established endometriosis and it is not always possible to carry out studies on the pathogenesis of this disease in humans. In addition, ethical considerations limit the performance of controlled experimental studies in humans, since it is not possible to monitor the progression of endometriosis without performing repeated laparoscopies. Thus, research investigating fundamental mechanisms by which menstrual endometrium adheres, invades, and establishes a functional vasculature to form endometriotic foci can be performed almost only in experimental animal models; moreover, studies on new therapeutic approaches are only possible in these models. Currently available medical therapies are unsatis-
factory, because they focus on relieving the symptoms rather than treating the disease. In addition, they cannot be used for prolonged duration because of severe secondary side effects [12]. This limitation is the main reason for research studies to develop specific and more efficient therapeutic alternatives that eliminate endometriotic lesions, prevent recurrences, and do not interfere with fertility potential.

Humans and non-human primates are the only mammals that spontaneously develop endometriosis; however, the limitations regarding human studies and the expense of primate studies have prompted investigators to pursue the use of small animal models [13]. Non-primate species used commonly for medical research do not menstruate and do not develop endometriosis; nevertheless, a number of investigators have mechanically introduced endometrial tissue into the peritoneal cavity of rabbits, rats, and mice in order to investigate multiple aspects of this disease. This type of study has proven to be valuable for studying some aspects of the disease, but surgical induction of endometriosis does not address the basic cellular mechanisms of endometrial attachment or invasion of ectopic lesions in women. The availability of implanting human endometriotic cells into experimental animals and inducing endometriotic lesions identical to those in women is an attractive approach for researchers who carry out endometriosis studies. Endometriosis-like lesions can be created by the transplantation of human endometrium into chicken chorioallantoic membranes [14], but these lesions can be maintained only short-term in developing embryos. Human endometrium has also been engrafted into the anterior chamber of the rabbit eye but these grafts begin to be rejected seven days after transplantation [15]. In contrast to humans and non-human primates, estrous animals do not shed their endometrial tissue and therefore do not develop endometriosis spontaneously. However, endometriosis can be induced by transplanting endometrial tissue to ectopic sites in these animal models, which are classified into two types: homologous and heterologous experimental animal models. In homologous models, which are immunocompetent animals, endometriotic lesions are induced utilizing the surgical transplantation of endometrium of the same animal or another syngeneic animal; whereas in heterologous models, which are immunodeficient animals, human endometrial fragments are directly transferred either intraperitoneally or subcutaneously. Although different experimental animal models have been described, the rat endometriosis model, which is a homologous model, has some advantages, such as limited costs; additionally, this model offers the opportunity to perform studies in large groups of genetically similar animals over a long period. It is well-suited for the investigation of mechanisms involved in the peritoneal attachment of endometrial cells as well as the investigation of the effects of therapeutic modalities. Moreover, it allows the evaluation of endometriotic foci at different time intervals and it is readily available in most experimental research centers [16]. In the present authors’ previous studies [4, 17], they used a homologous rodent model for the induction of endometriosis; however, since it was a homologous model it was not possible to induce endometriotic lesions using human endometriotic cells, which are identical to the endometriotic lesions in humans. Human endometrium and tissue samples from endometriotic lesions can be transplanted into immunodeficient mice [18, 19]. SCID mice have a combined immunodeficient T- and B-lymphocyte function and highly successful heterotopic transplantation was reported [5]. Immune-deficient nude mice lack a functional thymus and have a greatly reduced number of T lymphocytes but a normal complement of B cells. Greenberg and Slayden [20] subcutaneously engrafted human endometriotic tissue in transgenic recombinase-activating gene-2 knockout [RAG-2/γ(c)KO] mice, which are not only devoid of B and T lymphocytes but also lack NK cells. Phylogenetic differences between species can interfere with our understanding of endometriosis in humans, but the idea of implanting human endometriotic tissue into SCID mice and inducing human endometriosis is attractive and relevant. SCID mice offer the opportunity to create human endometriosis experimentally in a humanized animal model for endometriosis. SCID mice possess a congenital deficiency of B and T lymphocytes and thus cannot efficiently trigger either cellular or humoral immunity [21]. Aoki et al. [19] compared the take rate of human endometrium that was transplanted into nude and SCID mice and showed that nude mice rejected 60% xenografts, whereas 100% of the xenografts were accepted by SCID mice. It was shown that endometrial tissue can be implanted successfully on the peritoneum of nude mice [22]; however, Awwad et al. reported a higher success rate of implantation of 96.5% in SCID mice peritoneum [5]. In the present study, the authors demonstrated that human endometriotic tissue can be successfully implanted subcutaneously in SCID mice using an 18-G needle with a success rate of 100%. they also showed that the endometriotic lesions were responsive to melatonin treatment.

This is the first research project supported by the Scientific and Technological Council of Turkey (TUBITAK) carried out using SCID mice in which endometriotic lesions were induced by implanting human endometriotic cells. In the present study, the authors implanted endometriotic cells collected from 15 women with endometriosis subcutaneously in SCID mice in 15 sessions. In each session, cells were implanted in two SCID mice and these mice were randomized into treatment and control groups. Using this methodology, a representative sample of cells collected from different clinical endometriotic lesions was used to induce endometriosis in SCID mice. Subcutaneous implantation of human endometriotic cells in SCID mice makes it possible to remove the lesion sur-
ically without sacrificing the animal, so that the effects of hormones or other agents can be assessed over a longer study period. Since the present authors showed that melatonin was effective on recurrence of endometriosis in a homologous rat endometriosis model, they intend to carry out studies in the future using a heterologous animal model (SCID) and the same methodology used in the present study to evaluate the effects of some agents upon recurrence of endometriosis induced by implanting human endometriotic cells in SCID mice.

Because of side effects and high recurrence risk after cessation of treatment, there are ongoing investigations regarding new non-hormonal drugs for the treatment of endometriosis, which is a multifactorial disease. Oxidative stress was proposed as a potential factor in the pathophysiology of the disease [23]. Developing a better understanding of the cellular and molecular mechanisms linking endometrial biology to the pathophysiology of endometriosis remains an important aspect of the present authors’ studies; however, another aspect of an experimental disease model is testing the effectiveness of novel therapeutic agents. Therefore, new medical treatments aiming to reduce the oxidative stress with acceptable side-effects and as effective as hormonal treatment are needed. Melatonin seems to be promising in this sense. Melatonin is an endogenous free radical scavenger [24]. Although its mechanism is not clear, some of the steps in this mechanism are known. Melatonin can enter the cells easily because of its high diffusion ability; it can show its effects through its receptors and also without receptors, which makes it one of the most powerful antioxidants. In addition, melatonin may stimulate several anti-oxidative enzymes and inhibit a pro-oxidative enzyme by binding to calmodulin in the intracellular environment [25]. It is known that free radicals have a dual role in the reproductive tract and are key signaling molecules for endometriosis. Free radicals mediate their actions through a variety of pro-inflammatory cytokines, with these processes having been proposed as a common underlying factor for endometriosis. Superoxide anion (O$_2^-$) seems to be quite important in reactive oxygen species (ROS). Superoxide dismutase (SOD) rapidly decomposes superoxide anion into hydrogen peroxide and oxygen. Superoxide radicals are involved in many physiological and pathophysiological processes [26]. Malondialdehyde (MDA) can be found in most biological samples including foodstuffs, serum, plasma, tissues, and urine, as a result of lipid peroxidation, and has been reported widely for the purpose of estimating oxidative stress effects on lipids [27]. Melatonin is a documented powerful free radical scavenger and a broad-spectrum antioxidant [28]. It has been shown that melatonin causes regression and atrophy of endometriotic lesions in rats [4]. In the present study, the authors found that experimental endometriotic lesions induced in SCID mice, which has several advantages over other homolog experimental models, by using human endometriotic cells, could be used as a tool to test new therapies, such as melatonin, which is a potent antioxidant agent.

In conclusion, the SCID mouse is a promising heterologous animal model for experimental endometriosis studies to evaluate the etiology and pathophysiology of this disease, and to test the efficacy of some pharmaceutical agents on endometriotic lesions induced in this model that are identical to lesions in women. In the present study, melatonin treatment (20 mg/kg/d) was shown to be effective in the treatment of endometriosis induced in SCID mice using human endometriotic cells, in terms of histopathological scores and MDA levels; since possible side effects of this dose of melatonin in humans can be neglected to a large extent, the authors think that it may be high time to start carrying out clinical studies to evaluate the effects of melatonin on endometriosis in women. Due to results of the present study, the authors intend to make an application to the Ethical Committee of the Clinical Trials in order to able to carry out a study to test the efficacy of melatonin in human endometriosis.

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Address reprint requests to:
G. YILDIRIM, M.D.,
Yeditepe University Hospital,
Devlet Yolu, Ankara Cad. No:102-104.
34752, Kozyatagi, Istanbul (Turkey)
e-mail: gaziyildirim@gmail.com
Laparoscopic surgery improves pregnancy outcomes in women with suspected endometriosis with or without pathological confirmation


1 Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Greenville Health System, Greenville, SC (USA); 2 Department of Gynecology and Obstetrics, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS (Brazil)

Summary

Purpose of the investigation: To verify whether histologic confirmation of endometriosis impacts fertility outcomes. Materials and Methods: Women with unexplained infertility (UI) underwent laparoscopic excision or ablation with CO2 laser or electrocautery of all suspected endometriotic lesions, followed by clinical treatment between January 2007 and December 2013; pregnancy (> 12 weeks) within 12 months of monitored cycles was the main outcome measured. Results: Women with histological confirmation (n=74) did not differ from those not confirmed (n=29) with age, body mass index, gravidity, parity, ovulation induction protocol, and past duration of infertility. Pregnancy outcome was similar in both groups (39/74 vs. 15/29 - p = 0.9 – Chi-square) and there was no statistical difference in time to conceive/deliver (p = 0.7) between groups. Conclusions: There is no difference in fertility outcomes in women with UI, whether or not suspected endometriosis is confirmed pathologically.

Key words: Laparoscopy; Endometriosis; Pregnancy outcome.

Introduction

The 2002 National Survey of Family Growth stated that 2.1 million (7.4%) household women between the ages of 15 to 44 were infertile [1]. The diagnostic evaluation of infertile couples typically addresses five basic problems: the assessment of the male partner, documentation of ovulatory function, demonstration of patency of at least one fallopian tube, assessment of cervical and/or uterine disease, and assessment of intraperitoneal disease. The diagnosis of “unexplained infertility” (UI) is frequently applied to infertile couples where the male partner has normal sperm analysis and the female partner is ovulatory with at least one patent fallopian tube with a normal uterine cavity. The diagnosis of unexplained infertility occurs in up to 30% of couples [2]. Evaluation of peritoneal factors, such as pelvic adhesive disease or endometriosis, requires laparoscopic examination, a step that is increasingly delayed or omitted from the infertility workup, in favor of empirical treatment with in vitro fertilization (IVF), even though the utility of IVF for UI is not supported by evidence-based medicine [3]. The current European Society of Human Reproduction and Embryology (ESHRE) Endometriosis Consensus guidelines support the use of laparoscopy for minimal or mild disease, which is often the stage found in women with UI [4-10]. Others authors disagree with laparoscopy [11]. Badawy et al., in a large randomized clinical trial, concluded that laparoscopy could be postponed in unexplained infertility, until ovarian stimulation and timed intercourse were unsuccessful in achieving pregnancy [12]. In support of laparoscopy, Drake et al. reported that endometriosis was the dominant finding in patients with unexplained infertility [4] and endometriosis is associated with reduced fertility [13], even in its mildest forms [14, 15]. More recently, Littman et al. demonstrated an advantage to diagnostic laparoscopy for unexplained IVF failure patients, showing that most were able to conceive without IVF once endometriosis was identified and treated [16]. Phillips et al., evaluating cost effectiveness of surgery versus IVF for mild endometriosis, found that surgery was cost-effective compared to two cycles of IVF [17].

Surgeons treating infertility face a dilemma when their diagnosis of pelvic endometriosis is not confirmed by histological findings. Marchino et al., in a prospective clinical study, concluded that histological confirmation was necessary to validate the diagnosis of endometriosis, but they were unsure about the clinical impact of such findings [18]. Studies in the United States have implied that endometriosis is only diagnosed if lesions are confirmed by the pathologist [19], while a recent consensus by the ESHRE group on the diagnosis of endometriosis felt visual inspection was
Materials and Methods

Patient selection and variables

This cohort study was approved by the Institutional Review Committee of the Greenville Health System (Pro00013235, from September 3, 2013). The electronic records of all women who presented between January 2007 and December 2013 with UI at the Fertility Center of the Carolinas were reviewed for this study. All subjects met the following inclusion criteria: age between 21 and ≤ 41 years old, at least one year of infertility (defined as failure to conceive after one year of regular intercourse without use of any contraceptive method), a male partner with at least one normal sperm count who is able to father a child, an ovarian reserve suitable for IVF, at least 1 patent fallopian tube. Women with known intramural or subserosal fibroids, severe pelvic adhesions, endometrial polyps, factors of male factor infertility, who began clinical treatment in the next menstrual cycle.

Surgical groups

Subjects with visual evidence of endometriosis were further analyzed into two groups: those with or without histological confirmation of endometriosis. A board certified pathologist analyzed the biopsies and diagnosed endometriosis using standard histologic criteria [22]. Briefly, endometriosis was defined by the presence of endometrial glands and/or stroma with or without hemorrhage outside the uterine cavity. In some cases, pathological diagnosis was not obtained due to the paucity of disease, possible sampling error or due to cautery effect.

Outcomes

The primary outcome was crude viable pregnancy rate with up to one year of monitored cycles, in both groups. Viable pregnancy was verified by ultrasound and defined by the presence of intrauterine gestation(s) with fetal heartbeat in a gestation exceeding 12 weeks. Chemical pregnancy (presence of positive plasma or urine hCG, without ultrasonographic evidence of an embryo with heartbeat after five weeks of follow-up), and spontaneous abortion at ≤ 12 weeks were considered to be non-viable pregnancies. Cycle fecundity was calculated by dividing the number of viable pregnancies by the number of total cycles. In order to reduce bias, monitored cycle in the surgical group prior to surgery were excluded.

Sample size and statistical analysis

Sample size was calculated according to the data published by Nowroozi et al. [23] and Aanesen et al. [24], where pregnancy success after laparoscopic removal of endometriosis was 60%, and unexplained infertility treated with intrauterine insemination was 15%. With these figures and using an alpha error of 2.5% and a power of 95%, it would be required to have at least 28 patients in each group to have a 95% chance of detecting, as significant at the 2.5% level, an increase in pregnancy rates from 15% in the group of women without confirmation of endometriosis by pathology to 60% in those with pathological confirmation. Categorical variables, group and pregnancy outcome within 12 months, were compared by using the Log-rank (Mantel-Cox) Test. Demographic characteristics such as age, body mass index (BMI), gravidity, parity, and duration of infertility were compared by Student’s t test, if data had a Gaussian distribution, or Mann-Whitney analysis, if data did not have a Gaussian distribution. Normal distribution was calculated using D’Agostino & Pearson omnibus normality test.

Clinical treatments between groups were compared using Chi Square for trend analysis. Subgroup analysis was performed in subjects that had endometriosis under visual inspection. This group was subdivided into two groups: those who had confirmation by pathology report (pathology confirmed group), and those where endometriosis was not found (pathology not confirmed group). Fisher’s exact test was used in 2x2 tables. The Log-rank (Mantel-Cox) test was used to compare pregnancy outcome between the 12 months between these groups. Cases where visually suspected endometriotic lesions were not sent to pathology were considered in the pathology not confirmed group. Statistical calculation was performed with GraphPad Prism 6.0 software.
Laparoscopic surgery improves pregnancy outcomes in women with suspected endometriosis with or without pathological confirmation

Results

A total of 154 subjects were identified with UI between 2007 and 2013. From these, 108 met the inclusion criteria and were followed (Figure 1). Age, BMI, gravidity, parity, and duration of infertility were not different between groups (Table 1). Endometriosis was visually identified in 103 women and confirmed by histological examination in 74 cases (71.8 – 95%CI: 62.4 to 79.6). From all subjects with suspected endometriosis, 72.8% were assigned to rASRM Stage I or II (Table 1). Only four cases of suspected endometriosis did not have a biopsy sent to histological analysis (Figure 1). The average follow-up was three months and total was 12 months (range one to 12 months). Within 12 months of follow-up, successful pregnancy occurred in 39 of 74 of the confirmed group (52.7%, 95%CI 41.4 to 63.6), while in the group without pathological confirmation, successful pregnancy occurred in 15 of 29 (51.7%, 95%CI 34.4 to 68.6). There was no difference in the treatment types for ovulation induction between groups ($p = 0.07$; Chi-square for trend). There were no serious surgical complications reported in the laparoscopy group.

Table 1. — Demographics of the studied population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Confirmed (n = 74)</th>
<th>Not confirmed (n=29)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>32.4 ± 3.6</td>
<td>31.6 ± 4</td>
<td>0.3*</td>
</tr>
<tr>
<td>BMI</td>
<td>23.5 (20.4 to 25.7)</td>
<td>23.2 (20.6 to 25.9)</td>
<td>0.8*</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1 (0 to 1)</td>
<td>1 (0.5 to 1)</td>
<td>0.1*</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0 to 1)</td>
<td>0 (0 to 0)</td>
<td>0.2*</td>
</tr>
<tr>
<td>Months of Infertility</td>
<td>18 (12 to 30)</td>
<td>14 (12 to 24)</td>
<td>0.1*</td>
</tr>
<tr>
<td>Endometriosis stage n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (8)</td>
<td>11 (38)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>43 (58)</td>
<td>15 (52)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>18 (24)</td>
<td>2 (7)</td>
<td>0.0007c</td>
</tr>
<tr>
<td>IV</td>
<td>7 (9)</td>
<td>1 (3)</td>
<td></td>
</tr>
</tbody>
</table>

* Student t test – values are mean ± standard deviation;  
* Mann-Whitney test – numbers are median (25% and 75% percentile);  
* Chi-squared test for trend.

Table 2. — Cycle characteristics and therapies used by the study group.

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Not confirmed (n=29)</th>
<th>$p$ value</th>
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</thead>
<tbody>
<tr>
<td>Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cycles/patient (n)</td>
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<td>73/29</td>
<td></td>
</tr>
<tr>
<td>Pregnancies (n)</td>
<td>39</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>% per patients</td>
<td>52.7</td>
<td>51.7</td>
<td>0.9*</td>
</tr>
<tr>
<td>% per cycle</td>
<td>18.9</td>
<td>20.5</td>
<td>0.7*</td>
</tr>
<tr>
<td>Type of treatment (n)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Combined</td>
<td>93</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>2</td>
<td>7</td>
<td>0.07b</td>
</tr>
<tr>
<td>Oral</td>
<td>75</td>
<td>28</td>
<td></td>
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<tr>
<td>Superovulation</td>
<td>34</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher’s exact test;  
* Chi-square test for trend.

Figure 1. — Flowchart of the patients in the study.
Pregnancy outcomes were monitored in 279 cycles (206 cycles in confirmed group and 73 cycles in not confirmed group) (Table 2). Cycle fecundity in confirmed group was 18.9% (95%CI: 14.1 to 24.8%), while in the not confirmed group cycle fecundity it was 20.5% (95%CI: 12.8 to 31.1%). Using the Log-rank (Mantel-Cox) Test, the comparison between groups with or without pathological confirmation of suspicious lesions of endometriosis was not significantly different (Figure 2).

Discussion

There have been conflicting data between the ASRM and ESHRE guidelines on the impact of laparoscopy in patients with minimal and mild disease, as well as the need for confirmation of histological findings for the diagnosis of endometriosis. Since a lack of pathological diagnosis can occur for a variety of reasons unrelated to the pathophysiology of the disease or the impact of surgery, the present authors questioned whether confirmation should be required. In this study, they found that laparoscopic excision or ablation of suspected lesions of endometriosis yielded a 52% pregnancy rates after laparoscopy whether or not suspected endometriosis was confirmed pathologically.

Early prospective studies reported that endometriosis is present in 25 to 40% of women with infertility [25-28], but much has changed in the past 20 years with improved equipment and better appreciation of clear or subtle endometriotic lesions. Meuleman et al. suggest that women with normal ovulatory cycles, patent fallopian tubes, and normal male sperm counts, consistent with the present unexplained infertility group, have endometriosis in nearly 50% of cases [29]. The prevalence of pathology confirmed endometriosis in the unexplained infertility group was 71.8% (95%CI: 62.4 to 79.6), which is comparable to the data found by others [9, 27, 28, 30, 31].

As pointed out by the recent ESHRE guidelines, a positive histology confirms the diagnosis of endometriosis while negative histology does not exclude it [32]. Indeed, it could be argued that the biological impact of endometriosis is more important than the present authors’ ability to verify its presence histologically. There are a number of reasons why histological confirmation remains a poor indication of the true prevalence of endometriosis. Lesions may be ablated rather than sent to the pathologist. Cautery artifact may destroy the lesions before they can be read. Small lesions in the paraffin block section may not be included in a randomly selected cross-section. Microscopic disease including single cell layers may not be perceived as endometriosis by the pathologist. By monitoring pregnancy outcomes in women with endometriosis that did or did not have histological presence of disease, the present authors demonstrated that there was an increase in pregnancy rates, cycle fecundity, and time to pregnancy during laparoscopy that was not influenced by pathological confirmation.

In the present authors’ practice, they aggressively excised or ablated all suspected lesions, including rASRM Stage I which can have atypical, diffuse or subtle appearance. Despite this tendency to ablate lesions, the authors were able to confirm 74 out of 103 suspected endometriotic lesions (71%; 95CI = 62.4 to 79.6). Stegmann et al. recently reported that only 65% of suspected endometriosis lesions were confirmed histologically [19] in a large NIH-based study. The possible explanations for the present findings of benefit in lieu of confirmation likely are related to the alteration of immunological or inflammatory milieu [33]. Of note, the majority of lesions that were not confirmed by pathology were Stage I and II (Table 1).

The present data showing improvement in pregnancy rates is in accordance with the literature, including the EndoCan trial that examined the role of laparoscopy for minimal and mild disease, showing that fertility improved after ablation of lesions at laparoscopy [34-36]. The Canadian study required powder burn lesions in the inclusion criteria, which could explain why the improvement in fecundity was more modest than the present results. Red or opaque lesions have been shown to be biologically more active than powder burn implants [37]. One reason the present pregnancy results exceed that of the EndoCan study might be related to the fact that the authors routinely excised or ablated all red, clear or opaque lesions. As a further extension of the EndoCan study, this study suggests that pathological confirmation should not be considered essential for the diagnosis of endometriosis over visual inspection alone.
Strengths of this study include the large number of cases and the fact that most included samples sent for pathology examination. There is a need for this type of study, as very few investigations have been performed regarding whether or not pathology is required in order to predict outcomes. The present study suggests that minimal or mild endometriosis does contribute to infertility and that pathological confirmation does not change the outcomes. A limitation of this study was that it was not designed to show equivalence, but superiority; lack of difference does not prove equivalence. It would require over 1,082 cases to exclude a difference of more than 10% between both groups. Nevertheless, the present results are similar to those found in randomized clinical trials [36, 38]. The high incidence of endometriosis in the present sample reflects a careful selection of subjects with prolonged unexplained infertility and signs and symptoms of endometriosis. Given today's diagnostic workup that includes semen analysis, hysterosalpingography and sonohysterography, endometriosis is a diagnosis of exclusion and is not an unexpected finding in this group of patients. The authors did not include monitored cycle prior to surgery, as this would have biased the data analysis, since 100% of these patients had failed to conceive prior to surgery.

Conclusion

Histological confirmation does not appear to be essential in order to demonstrate benefit of laparoscopic excision or ablation of suspected lesions of endometriosis. With better diagnostic tests that reliably predict the presence of endometriosis will likely facilitate future studies that seek to better define the relationship between minimal endometriosis and infertility.

Acknowledgements

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References


Address reprint requests to:
B.A. LESSEY, M.D., Ph.D.
890 W. Faris Rd, Suite 470,
Greenville, SC 29605 (USA)
e-mail: blessey@ghs.org
Introduction

Amniotic band syndrome (ABS) has an international incidence that ranges from 1:1,200 to 15,000 births without a preference for sex or race. In Latin America, the reported prevalence is 1:11,200 births [1-3]. ABS is a set of fetal malformations that are caused by fibrous adhesions (amniotic bands), which trap and strangle fetal parts, such as the extremities and can cause ischemic damage to the tissue distal to the bands. This phenomenon can result in hydropenia, amputation, syndactyly, and polydactyly. When the amniotic fluid is ingested and swallowed by the fetus, the fetal development of the face can be disrupted, leading to craniofacial malformations and a cleft palate. Other malformations that have been described include anencephaly, encephalocele, ectopia cordis, and a ventral diaphragmatic defect [1-3].

There are two theories on the mechanism of fetal malformations in this disease. The endogenous theory states that the defects that are associated with ABS occur in response to fetal development complications in the formation of connective tissue [4]. The exogenous theory was proposed by Torpin in 1968 and states that the rupture of the amnion, specifically when the chorion remains undamaged, causes a transitory oligohydramnios secondary to the loss of amniotic fluid through the chorion. The fetus passes from the amniotic cavity to the chorionic cavity via this defect. The contact between the fetus and the “adherent” mesoderm of the chorionic surface of the amnion leads to tangling of fetal parts and cutaneous lesions, which have been observed in cases of ABS [5].

Animal research has provided evidence that a puncture or rupture of the amnion disrupts the embryological vessels during development, which causes ischemia and injuries, such as hypoplasia and the amputation of the extremities [6, 7]. Following the Kino model, the rabbit can be used as an animal model for the prenatal development of amniotic bands due to the number and diversity of anomalies that ABS presents [8].

The objective of this study was to determine whether puncturing the uterine wall and the amnion causes uterine contractions, which are associated with different types of fetal abnormalities.

Materials and Methods

In accordance with the Official Mexican Standard NOM-062-ZOO-19998 [9], all procedures were approved by the Research and Ethics Committee of the Dr. Manuel Gea Gonzalez General Hospital and the Institutional Committee for the Care of Laboratory Animals (ICCLA). The design of this study was comparative, open, experimental, prospective, and transverse.

A total of 12 female New Zealand rabbits that weighed three to 3.5 kg were housed in individual cages. A female was brought to the male cages for mating, which was verified by the veterinarian and zootechnician, and this day was considered day 0. Four groups of three rabbits were formed. The uterine wall and amniotic membranes of one of the uteri was punctured in group A on day 15 of gestation followed by group B on day 16, group C on day 17, and group D on day 18. The duration and force of contractions and fetal abnormalities were determined. Results: There were immediate contractions after the puncture, which lasted 20 to 132 seconds with forces that ranged from 309 to 4,411 mg. All of the experimental fetuses exhibited anomalies of the head and extremities, exencephaly, cleft palates, and an absence of eyelids. Conclusion: Injury to the uterine wall and the amnion can immediately cause uterine contractions, which are associated with different types of fetal abnormalities.

Key words: Amniotic band syndrome; Fetal abnormalities; Therapy.
hours). Enrofloxacin was administered at ten mg/kg (IV), and a 2.5-mm (internal diameter) endotracheal tube was inserted via the nose with a previous instillation of 2% lidocaine. Once fixed, the tube was connected to the anesthesia circuit for the passage of isoflurane at 5% over three minutes for induction and at 3% for maintenance during the surgery.

During the laparotomy, a trichotomy was performed, and the abdominal wall was cleaned with 0.7% iodine and 74% isopropyl alcohol. An abdominal midline incision was made, and the uteri that were closest to the ovaries (right and left) were identified. The uterus of the doe is formed by two horns right and left and each horn has a cervix that opens into the vagina. You can have on average four uteri and each uterus behaves as an independent unit. With its monochorial placenta, amniotic sac, and the uterine wall placenta.

The normal period of gestation in the doe is on average 31 days in 98% of cases, but can vary from 29 to 35 days, depending on the number of fetuses in gestation (varies from four to 12), to minor number of fetuses the greater the period of gestation and vice versa. One of the wombs (experimental) was randomly chosen for the physiological study, in which an "S" hook was placed on the wall of the uterus and a baseline recording of contractile activity was obtained as a control. The wall of the uterus and the amniotic membranes were punctured using an insulin syringe with a 0.2-mm needle (outer diameter) that avoided blood vessels and prevented injury to the fetus. A single puncture was performed regardless of whether amniotic fluid was extracted; uterine muscle activity (pitch and duration) was immediately recorded over 240 seconds (the time when contractions appeared, which had previously been standardized with two female rabbits per group). A digital data acquisition system and the Biopac Student Lab PRO (v. 3.7) were used to obtain the recordings, which were later analyzed offline. The contralateral uterus that was closest to the ovary was used as a control, those who took the baseline uterine tone control and no puncture was performed.

After the procedure, the abdominal wall was closed at two levels: the muscle was closed with Vicryl 2-0, and the skin was closed with Prolene 3-0. During the postoperative period, two mg/kg of flunixin meglumine was administered intramuscularly every 12 hours for three days, and ten mg/kg of enrofloxacin was administered intramuscularly every 12 hours for six days.

On day 29 of gestation, the female rabbits and the fetuses were sacrificed with a sodium pentobarbital overdose. The fetuses in the uterus, control, experimental, and remaining uteri were examined macroscopically. The uteri were identified (experimental and control) and extracted with the products of the uterus. The uteri were infiltrated with formalin, and a macroscopic examination of the fetuses was conducted after eight days.

The following variables were analyzed: the day of gestation, the quantity of fluid amniotic extracted, fetal deformities, the force of the contractions as measured in mg, the number of contractions in 240 seconds, and the duration of the contractions as measured in seconds. The variables were analyzed using the following descriptive statistics: the mean, the standard deviation (SD), and the range.

Results

The punctures were performed on 12 uteri, three per group. In the punctured uteri of group A (punctured at 15 days of gestation), there was an average of three contractions in 240 seconds. The average duration of the contractions was 56.4 seconds with a range of 36-80 seconds and a SD of 14.62 seconds. The average basal uterine tone measured before the puncture was 125.8 mg, and the average force of the contractions was 497.3 mg with a range of 309.3 to 909.2 mg and a SD of 216.52 mg (Table 1).

During the macroscopic examination of the three experimental fetuses, one abortion, one death, and one fetus with exencephaly, an absence of eyelids, a cleft palate, and limb compression were observed (Figure 1). Among the three control fetuses, one abortion occurred. In the remaining fetuses, one death, one abortion, one forelimb compression, and one compression of the facial area occurred.

In group B (punctured at 16 days of gestation), an average of three contractions was observed in 240 seconds. The average duration of the contraction wave was 63.2 seconds, with a range of 38-120 seconds and a SD of 26.57 seconds. The average basal uterine tone was 178.9 mg, and the average force of the contractions was 997.1 mg with a range of 434.4 to 2,059.4 mg and a SD of 532.83 mg (Table 2). The anomalies that were found in the three experimental fetuses included cephalic compression and two deaths (Figure 2). In the three control fetuses, a limb deformity was observed. In the remaining fetuses, there was one fetus with exencephaly, eight fetuses with cephalic compression, two fetuses with clubfoot, eight limb deformities, and four deaths.

In group C (punctured at 17 days of gestation), there was an average of three contractions in 240 seconds. The average duration of the contraction wave was 51.6 seconds with a range of 20-84 seconds and a SD of 23.5 seconds. The average basal uterine tone was 192.4 mg, and the average force of the contractions was 1,936.5 mg with a range of 661.2 to 3,189.1 mg and a SD of 742.4 mg (Table 3). The anomalies that were found in the three experimental fetuses included limb and tail deformities and two deaths (Figure 3). In the three control fetuses, a deformity of the right hind limb was found. In the remaining fetuses, there were six fetuses with cephalic compression, six limb deformities, four fetuses with clubfoot, and one death.

In group D (punctured at 18 days of gestation), there was an average of three contractions in 240 seconds. The average duration of the contractions was 56 seconds, with a range of 24-132 seconds and a SD of 36.15 seconds. The average basal uterine tone was 261.1 mg, and the average force of the contractions was 2,035.4 mg with a range of 957.6 to 4,411.5 mg and a SD of 1,195.74 mg (Table 4). In the three experimental fetuses, the following abnormalities were found: one case of cephalic compression, one case of exencephaly, two forelimb deformities, three cases of clubfoot, and two tail deformities (Figure 4). In the three control fetuses, one case of cephalic compression was observed. In the remaining fetuses, there were seven fetuses with cephalic compression, four forelimb deformities, nine hind limb deformities, and eight tail deformities. An average of 0.3 ml of amniotic fluid was extracted from ten of the 12 punctured uteri.
Table 1. — Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 15 days of gestation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration of the contractions in seconds</th>
<th>Force measurements of contractions (mg)</th>
<th>Anomalies found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st contraction</td>
<td>2nd contraction</td>
<td>3rd contraction</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

Range 36-80
Mean 56.4
*SD 14.62

Table 2. — Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 16 days of gestation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration of the contractions in seconds</th>
<th>Force measurements of contractions (mg)</th>
<th>Anomalies found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st contraction</td>
<td>2nd contraction</td>
<td>3rd contraction</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>38</td>
<td>48</td>
</tr>
</tbody>
</table>

Range 38-120
Mean 63.2
*SD 26.57

*SD = standard deviation.

Figure 1. — An experimental fetus from group A (at 15 days of gestation): (a) exencephaly, (b) an absence of eyelids, and (c) a cleft palate.
Table 3. — Time and force measurements of uterine contractions and the abnormalities in fetuses from uteri that were punctured at 17 days of gestation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Amount of amniotic liquid extracted (ml)</th>
<th>Experimental</th>
<th>Control</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deformity of the forelimb, hind limb, and tail</td>
<td>None</td>
<td>3 fetuses with cephalic compression, 3 fetuses with a forelimb deformity, and 2 fetuses with a bilateral clubfoot</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Death</td>
<td>Deformity of a hind limb</td>
<td>1 fetus with cephalic compression, 1 fetus with compression of the left forelimb, and 1 fetus with a clubfoot</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Death</td>
<td>None</td>
<td>1 death, 2 fetuses with cephalic compression, 2 fetuses with a deformity of the right forelimb, and 1 fetus with a bilateral clubfoot</td>
<td></td>
</tr>
</tbody>
</table>

*SD = standard deviation.

Figure 2. — Experimental fetuses from group B: (a) cephalic compression and (b) fetal death.
Discussion

To date, the pathogenesis of ABS is unclear. Several studies have associated ABS with a history of maternal trauma, oophorectomy during pregnancy, the use of intrauterine devices, and invasive procedures, such as amniocentesis. Amniotic bands do not increase maternal risk during pregnancy; however, these bands generally cause premature labor [10] and can result in numerous irreversible and severely disfiguring fetal injuries that are incompatible with life [11, 12]. However, no study has demonstrated that uterine contractions can cause ABS anomalies.

In the present study, the authors found that after puncturing the wall and amniotic membranes of rabbit uteri using a fine needle and without injuring the fetus, uterine contractions immediately occurred and lasted between 20 and 132 seconds. These contractions generated changes in the fetal environment, which resulted in a variety of abnormalities, such as exencephaly and limb deformities. The contraction force increased from a baseline average of 189.6 mg to at least 309.32 mg (1.6-fold increase) and up to a maximum of 4,411.51 mg (22-fold increase).

There was an association between the increase in the force of the contractions and the day of gestation, which began with an average force of 497.3 mg at 15 days of gestation and increased up to 2,035.46 mg of force at 18 days of gestation. This finding indicates that the strength of the uterine contractions increased with the day of gestation.

All the experimental fetuses had an anomaly, such as exencephaly, a cleft palate, an absence of eyelids, cephalic compression, a clubfoot, a limb deformity, death, and abortion. Previous data indicated that there is a direct relationship between the contractions and the anomalies that are found.

As discussed by Kino [7], uterine contractions can cause decreased blood flow from the placenta to the embryo, which results in a type of injury that depends on the gestational age. The present authors hypothesize that the different anomalies that we found were associated with the following three factors: the force and the duration of the contractions, the day of gestation, and the loss of amniotic fluid. On days 15 and 16 of gestation, the injuries were more evident, such as exencephaly, an absence of eyelids, a cleft palate, death, and abortion. On days 17 and 18, the authors found skull, limb, and tail compressions and cases of clubfoot.

Nakayama et al. observed that uterine and fetal surgery procedures on rhesus monkeys between 123-152 days of gestation, such as amniocentesis, maternal laparotomy without uterine manipulation, and hysterectomy with and without fetal surgery, induced patterns of electromyographic activity (EMG) and that the uterine contractions were similar to the contractions that are observed during preterm delivery. A characteristic of this response is bursts in EMG activity, called type I [13].

The study was based on the study of Kino [7] that mentions that uterine contractions, decrease blood flow from the placenta to the embryo, which causes lesions in the extremities, but he only made histopathological studies, and the present study measured electrographically uterine contractions. If it intends that uterine contractions may be an important factor in the presence of SBA, it is independent of the theories already described for its origin as the endogenous and exogenous.

In the ABS: these bands occur when uterine contractions break the amnion forming amniotic bands that strangle extremity or in case of being swallowed by the fetus caused cleft palate. Amniotic bands do not necessarily have to exist to cause anomalies; uterine contractions can not break the amnion and compression of tissues of the fetus can lead to another series of severe anomalies.

According to the above described, there can or cannot be the loss of the amniotic liquid, and when there are contractions lesions may be more severe. Rather, these have been described in the context of the amnion ruptura sequence (ARS) in previable fetuses, as reported by Kalousek and Bamforth [14].
Other questions should be answered in future studies, such as whether uterine contractions cause a decrease in blood flow from the placenta to the fetus and whether substances or mediators are released before the stimulus that causes uterine contractions.

The results of this study may explain anomalies that occur in children conditioned by some external stimulus caused to the mother during the first and second trimester of pregnancy in this study caused by uterine contractions. Which would lead us to consider preventive measures.

Conclusions

The present authors identified mechanisms that are involved in the anomalies present in ABS and differ from the mechanisms of the previously proposed theories (endogenous and exogenous). Uterine contractions were the main factor in these mechanisms.

The results of this study could explain the anomalies caused by external agents during second trimester of gestation; those agents would begin the uterine contractions.

Other questions should be answered in future studies, such as whether uterine contractions cause a decrease in blood flow from the placenta to the fetus and whether substances or mediators are released before the stimulus that causes uterine contractions.

References


Address reprint requests to:
G. MONTAÑO ALFONSO, M.D.
General Hospital “Manuel Gea González”
Calzada de Tlalpan 4800, Colonia Sección XVI.
Del. Tlalpan, Distrito Federal (Mexico)
e-mail: gamagq3@hotmail.com
Predictive value of biochemical marker ADAM-12 at first trimester of pregnancy for hypertension and intrauterine growth restriction

C. Kasimis¹, N. Evangelinakis¹, M. Rotas¹, M. Georgitsi², N. Pelekanos², D. Kassanos¹

¹ 3rd Department of Obstetrics and Gynaecology, Medical School of Athens, General University Hospital “Attikon”, Athens
² 4th Department of Internal Medicine, Medical School of Athens, General University Hospital “Attikon”, Athens (Greece)

Summary

Purpose: Delineate whereas ADAM-12 levels at first trimester of pregnancy may be used as a marker for hypertension-preeclampsia (PE) and intrauterine growth restriction (IUGR). Materials and Methods: The present is a case control study. Serum ADAM-12 of women presenting for routine assessment of risk for chromosomal abnormalities at 11+0 to 13+6 weeks of gestation was measured. The study group comprised of 98 pregnancies that subsequently developed pregnancy-induced hypertension (PIH) or PE or small for gestational age fetuses (SGA), and were compared to 100 uncomplicated pregnancies. Results: There was no statistically significant difference of mean log multiple of the expected median (MoM) of ADAM12 between control group and the group that consisted of all women with complicated pregnancy (PE, PIH, and SGA). ADAM-12 levels in women who developed PE during pregnancy were significantly lower than in women of control group (mean log MoM: 0.109 vs. 0.008, p = 0.010). Similarly, ADAM-12 levels in women who developed PE and/or PIH were significantly lower than in women of control group (mean log MoM: 0.066 vs 0.008, p = 0.015). There was no significant difference of ADAM12 levels between controls and pregnancies with SGA fetuses. Conclusion: Maternal serum levels of ADAM-12 at the first trimester are significantly lower in women who later develop PE when compared with women with uncomplicated pregnancies.

Key words: ADAM-12: Preeclampsia; Hypertension; Biochemical marker.

Introduction

Preeclampsia (PE) affects about 2% of pregnancies and remains a major cause of perinatal morbidity and mortality. The underlying pathophysiology for PE is thought to be impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries, and this hypothesis is confirmed by histological and Doppler studies of uterine arteries [1]. Hypertension during pregnancy is distinguished in pregnancy-induced hypertension (PIH) and pregnancy with hypertension accompanied with proteinuria that is called PE. PE is further distinguished in early (<34 weeks of gestation) and late PE (>34 weeks of gestation) [2].

Etiology of PE is multifactorial and remains basically unknown. Genetic predisposition, impaired placentation, immunological disorders, and pre-existing hypertension of the background of hypertension-PE. Over the last decade research has focused on identifying biochemical markers that could predict early on in pregnancy the onset of PE [3].

Biochemical marker ADAM-12 (a disintegrin and metalloproteinase) is associated with intrauterine growth restriction (IUGR), PE, and chromosomal abnormalities [4-6]. ADAM-12 is produced by the placenta and is a part of the ADAM proteins’ group, which consists of more than 15 members. ADAM proteins play a key role in adhesion and apoptosis of cells.

In humans ADAM-12 is found in two forms. The soluble ADAM-12 (sADAM-12) is expressed in placenta, whereas ADAM-12-L is expressed in several different tissues. ADAM-12 exerts a proteolytic action on growth factors IGFBP-3 and IGFBP-5 and participates in regulation of action of insulin- growth factors, such as PAPP-A [7, 8]. Disintegration of IGFBP-3 stimulates growth of tissues and consequently non-disintegration of IGFBP-3 is associated with restriction of growth. Biochemical markers that have been associated with hypertension and PE are PAPP-A, β-hCG, placental growth factor (PIGF), PP13, and anti-angiogenic protein soluble FMS-like tyrosine kinase-1 [sFlt-1] [9].

In the first trimester, uterine artery Doppler pulsatility index [PI] alone has a sensitivity of around 20-30% for the detection of PE. This sensitivity is better for the prediction of early PE (40-50%), but this is improved by the addition of measurements of maternal serum biochemistry [10].

Small for gestational age (SGA) are considered the fetuses whose growth and weight is beneath the 10th percentile adjusted for the gestational age. SGA fetuses are distinguished by those that are constitutionally small due
to genetic predisposition, those whose growth is restricted, have abnormal Doppler flow in the vessels, and are called IUGR fetuses. In IUGR fetuses there is usually abnormal flow in umbilical artery (increased resistance) and abnormal Doppler flow in middle cerebral artery (low resistance-low PI-redistribution) and later in the ductus venosus when hypoxia has established absent or reversed flow. The first step is the distinction of true IUGR fetuses, associated with signs of abnormal fetoplacental Doppler flow from constitutionally SGA fetuses that have a normal Doppler measurements and perinatal outcome.

IUGR is associated with a poorer perinatal outcome, including impaired Doppler cerebro-placental ratio and an estimated fetal weight (EFW) below the 3rd centile. Once the diagnosis is established, differentiating into early- and late-onset IUGR is useful and distinguishes two quite different clinical situations concerning perinatal outcome and onset of PE [11-15].

The aim of the present study is to delineate where the biochemical marker ADAM-12, measured in maternal serum during the first trimester of pregnancy, could be used as an early predictor for hypertension-PE and IUGR.

Materials and Methods

The present is a case control study. Screening was performed in pregnant women that presented in the Fetal – Maternal unit of the 3rd Department of Obstetrics & Gynaecology of Attikon University Hospital for routine assessment of risk for chromosomal abnormalities by measurement of fetal nuchal translucency thickness and PAPP-A and free β-hCG at 11+0 to 13+6 weeks of gestation. Gestational age was determined by sonographic measurement of fetal crown-rump length (CRL).

The authors obtained a thorough medical history which included several maternal characteristics and subsequently blood was drawn. The serum harvested from whole blood was stored at -80°C for future biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study which was approved by Ethical committee of “Attikon” University Hospital.

The study population comprised of 98 pregnancies that subsequently developed PIH or PE or SGA fetuses – neonates, and 100 uncomplicated pregnancies, that delivered appropriate for gestational age and phenotypically normal neonates, as control group.

All women are of Greek origin and none of them had history of hypertension. In those pregnancies that were complicated by SGA maternal characteristics and subsequently blood was drawn. The serum harvested from whole blood was stored at -80°C for future biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study which was approved by Ethical committee of “Attikon” University Hospital.

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All women are of Greek origin and none of them had history of hypertension. In those pregnancies that were complicated by SGA neonates (SGA group), PE (PE group) and PE and/or PIH (PE and/or PIH group) maternal serum concentration of ADAM-12 was measured at 11+0 – 13+6 weeks of gestation. SGA group included pregnancies in which birth weight of neonates was below the 10th percentile adjusted for gestational age. Serum ADAM-12 was measured by a quantitative enzyme–linked immunosassay technique using two kits (human ADAM 12–immunoassay, Lot 306455, Lot 312925). Each sample was diluted twice and results of ADAM-12 are expressed in ng/ml.

**Statistical analysis**

Median ADAM-12 concentrations were obtained by the antilogarithm to the predicted ADAM12 values as obtained from linear regression analysis of the logarithm of ADAM-12 by gestational days in the control group. Afterwards all ADAM-12 measures were expressed as multiple of the expected median (MoM) in the control group. The logarithm of MoM was compared between the study groups.

Categorical variables are presented as absolute and relative frequencies. Quantitative variables are presented with mean and standard deviation (SD) or with median and interquartile range (IQR). Chi square and Fisher’s exact tests were used for the comparison of proportions between the study groups. When the normality assumption was satisfied, the Student’s t-test was used for the comparison of means of continuous variables between two groups and the Mann-Whitney test when the distribution was not normal. All p values reported are two-tailed. Statistical significance was set at 0.05 and analyses were conducted using SPSS (version 19.0).

**Results**

ADAM-12 was measured in 100 normal pregnancies and 98 complicated pregnancies. Demographics and clinical characteristics for both groups are shown in Table 1. The two groups were similar in terms of maternal age, BMI, smoking, conception, number of previous pregnancies, and gestational age at the time of ADAM-12 measurement. Eight women

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**Table 1. — Demographics and clinical characteristics of the control and pathological groups.**

<table>
<thead>
<tr>
<th></th>
<th>Control n=100</th>
<th>Pathological n=98</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, mean (SD)</td>
<td>35.1 (5.0)</td>
<td>35.4 (6.5)</td>
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</tr>
<tr>
<td>Racial origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greek</td>
<td>100 (100)</td>
<td>98 (100)</td>
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<td>26 (3)</td>
<td>26.1 (4.7)</td>
<td>0.844‡</td>
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<td>Smoking</td>
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<tr>
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<tr>
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<td>7 (7)</td>
<td>7 (7.1)</td>
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<td>0.542**</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>2 (2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks), median (IQR)</td>
<td>12 (11.0-12.2)</td>
<td>12 (11.0-12.1)</td>
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<td>Birth weight, median (IQR)</td>
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<td>2100 (1950-2500)</td>
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<td>75 (76.5)</td>
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†Student’s t-test; *Chi-square test; **Fisher’s exact test; △Mann-Whitney test.
Predictive value of biochemical marker ADAM-12 at first trimester of pregnancy for hypertension and intrauterine growth restriction

(8.2%) in the complicated group had diabetes. Birth weight and gestational age at birth were significantly lower in that group. Seventy-five infants (76.5%) in the complicated group were classified as SGA. In that group 43 (43.9%) women had PE and/or PIH and 15 (15.3%) women had only PE.

Table 1 and 2 present the demographics and clinical characteristics for SGA, for women with PE, and women with PE and/or PIH. The SGA group, the PE group, and the PE and/or PIH group were similar when compared to the control group in terms of maternal age, BMI, smoking, number of previous pregnancies, and gestational age when ADAM-12 was measured. Diabetes was more frequent in the SGA group. IVF was more frequent in the PE group and the PE and/or PIH group and as expected the birth weight and gestational age at birth were significantly lower in the aforementioned groups and the SGA group as compared to the controls.

Mean log MoM of ADAM12 for all study groups are described in Table 3. There was no statistically significant difference of mean log MoM of ADAM-12 between control group and the group that consisted of all women with complicated pregnancy (regardless if it was PE, PIH or SGA). On the contrary, ADAM-12 levels in women who developed PE during pregnancy had a mean log MoM of -0.109, which was significantly lower than the mean log MoM of 0.008 for ADAM12 levels observed in samples from women of the control group ($p = 0.010$). Similarly, ADAM-12 levels in women who developed PE and/or PIH during pregnancy had a mean log MoM of -0.066, which was significantly lower than the mean log MoM of 0.008 for ADAM12 levels observed in samples from women of the control group ($p = 0.015$) (Figure 1). Furthermore, no significant difference was found in mean log MoM of ADAM-12 when compared between pregnancies with SGA infants and controls.

Discussion

ADAM-12 is a protease with important role in muscle development and neurogenesis [16]. The gene of ADAM-12 produces two different transcripts: one long form attached to...
the membrane, and one shorter that is soluble. Concentration of ADAM-12 is studied in many different conditions during pregnancy and is found to be altered when pathology is involved. Specifically, maternal serum concentrations of ADAM-12 are lower in pregnancies of fetuses with trisomies, such as 18 and 21 [17]. Similar results are reported, also, in pregnancies with other aneuploidies and SGA fetuses [18].

ADAM-12 is a biological marker that has been studied in chromosomally abnormal and IUGR fetuses, and less extensively in PE. The present study shows that women that eventually developed PE and/or hypertension during pregnancy had statistically significantly lower levels of ADAM-12 during the first trimester. The same statistical significance was revealed when women only with PE were studied and compared to the control group. No statistical significance in ADAM-12 serum levels was revealed among women with SGA neonates and the control group. The present results seem to be in accordance to the conclusions presented by other research groups, such as Laigaard et al., el-Sharbiny et al. [4-7]. In particular, Laigaard et al. reported significantly lower concentrations of ADAM-12 in the first trimester in a population of 160 women with PE compared to 324 healthy women with uncomplicated pregnancies [4]. Spencer et al. reported that ADAM-12 can be used as a marker for chromosomal abnormalities [17].

Some research groups published less promising results. Poon et al. report that measurements of ADAM-12 concentrations at first trimester do not predict SGA and PE [19].

Up-to-date studies indicate that ADAM-12 may have a role in cellular activity and development. ADAM-12-L, that is mainly located at the cellular membrane, as well as the soluble form (s-ADAM-12), are supposed to be produced by trophoblasts. Some researchers support that ADAM-12 contains receptor areas, like integrins, and this is the manner in which they affect differentiation and cellular survival.

Placentas of pre eclamptic women appear to express higher levels of adhesive substances, such as integrins. It is possible that decreased levels of ADAM-12 affect the time required for differentiation and development of the cells.

Previous studies indicate that ADAM-12 disintegrates IGFBP-3 and IGFBP-5, and it is known that the function of IGF axis is crucial for the development of PE. IGFBP-3 appears to have inhibitory effect on the development of cells. In case of lower levels of ADAM-12, IGFBP-3 will not disintegrate and development of cells is inhibited [20]. Other researchers support that ADAM-12-L interacts with heparin-binding–epidermal growth factor, with placenta leukine aminopeptidase and with other growth factors [21].

Thus, in order to develop a complete screening tool for PE it is important to combine evaluation of risk factors (such as genetic predisposition, history of PE in previous pregnancy, etc.) along with biochemical markers (such as PAPP-A, inhibin, PLGF, ADAM-12) and Doppler measurements at first trimester. False positive results will be significantly decreased and early detection of high-risk women for developing PE will give the chance for early intervention and therapy [11].

**Conclusion**

Maternal serum levels of ADAM-12 are significantly lower during the first trimester in women who later develop PE during pregnancy when compared to the levels of women with normal pregnancies. Thus, ADAM-12 could be a useful prediction marker for PE, especially when used in combination with other biological markers or Doppler measurements. Maternal serum levels of ADAM-12 are non statistical significantly different during the first trimester in women who later had fetuses SGA when compared with levels of ADAM-12 in normal and uncomplicated pregnancies.

**References**

Predictive value of biochemical marker ADAM-12 at first trimester of pregnancy for hypertension and intrauterine growth restriction


Address reprint requests to:
C. KASIMIS, M.D.
3rd Dept. of Obstetrics and Gynecology
University of Athens, “Attikon” Hospital
Rimini 1, Chaidari,
Athens 12462 (Greece)
e-mail: h.kasimis@hotmail.com
Pregnancy after heart surgery – challenges

S. Plešinač, I. Pilić, I. Babović

1 Medical School University of Belgrade, Belgrade; 2 Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade (Serbia)

Summary

Purpose: Advances in cardiac surgery significantly improve life expectancy and quality of life of patients with congenital or acquired heart disease. Materials and Methods: The present study included 146 pregnant women who had antenatal care and gave birth at the Institute of Gynecology and Obstetrics in an interval of ten years from 1994-2004. Patients were divided in four groups according to the type of heart surgery. Group I included four patients with surgically corrected coarctation of the aorta. Group II included 27 patients with correction of congenital heart diseases, and group IV had 47 patients with artificial heart valves. Results: There was 4% of heart failure after delivery. The incidence of hemorrhagic complications during pregnancy was 2.7% and 4.1% after delivery. The incidence of thromboembolic complications after delivery was 6%. Four newborns died, one of hydrocephalus and three of hypoxic ischemic encephalopathy. Two patients died. Conclusion: Patients with artificial heart valves need an enhanced level of medical care during pregnancy and labor.

Key words: Congenital heart disease; Heart surgery; Pregnancy; Anticoagulation therapy.

Introduction

Advances in cardiac surgery improve quality of life and level of functioning of cardiovascular system of patients with congenital or acquired heart disease. These diseases complicate from 0.1% to 4% of pregnancies. Half of them are congenital. Maternal mortality of patients with New York Heart Association (NYHA) Functional Status class III and IV is 8% [1]. Congenital heart disease includes anatomic as well as functional defects of cardiovascular system, which are the result of incorrect embryogenesis. The reasons are still unknown but infections, immunological disorders, genetics, and medications are possible risk factors. The inheritance rate is 2-10%.

In congenital heart disease heart surgery can be performed in childhood, but also any time in life, even during the pregnancy. Important point for these patients is coordinated care of cardiologist, surgeon and obstetrician in order to monitor the pregnancy and to determine the risks for mother and for fetus and minimize them.

Special group of patients are those with artificial heart valves. They receive continuous anticoagulation therapy. Therefore they are an extreme challenge for the obstetricians. Heart disease and hemodynamic changes during pregnancy place a special burden on underlying heart disease. Complications resulting in maternal death are thromboembolic, hemorrhagic, and heart failure. The fetus is in danger of hypoxia, genetics and oral anticoagulation therapy, and other medications given to the patient in order to support cardiovascular system.

The aim of the study was to analyze course and outcome of pregnancy in patients after surgical correction of congenital or acquired heart disease. Answers to many questions are still unknown. When can we advise the patient to continue the pregnancy despite the risks for mother and fetus? What anticoagulation and other therapy should be given to the patient? Which medications are safe for fetus? How frequent should these women be monitored? If necessary, what is the most appropriate time and way to terminate a pregnancy? How does the pregnancy influence the heart disease and in what way does the disease influence mother and fetus? Are all patients who had heart surgery at the same risk during pregnancy?

Materials and Methods

The present study included 146 pregnant women who had antenatal care and gave birth at the Clinic of Gynecology and Obstetrics in an interval of ten years (1994-2004). The study was prospective. The same team of obstetricians and cardiologists followed the patients. The changes in therapy principles during these years were carefully applied, especially anticoagulation therapy. Patients were divided in four groups according to the type of heart disease and the type of surgery. Group I included four patients with surgically corrected coarctation of the aorta. Group II included 27 patients with correction of congenital heart diseases and group IV had 47 patients with artificial heart valves. Group IV was divided in two subgroups: A - 22 patients under oral anticoagulation therapy and B - 25 patients who received heparin in the last four weeks of pregnancy and after delivery. Oral anticoagulation therapy was ethyl biscumacetate.
All patients had regular hematological and biochemical controls every two weeks. These included: hemoglobin levels, hematocrit, red blood cells, platelets, coagulation time, and APTT or INR testing depending on the type of anticoagulants received. Fetal evaluations were made every three to four weeks, and they included ultrasound examination and measurements, and biophysical profile. Labor was induced only for obstetric indications. Oxytocin was used for induction of labor. However, it was administered cautiously in a concentrated solution to avoid water overload. All patients received antibiotics for prophylaxis against infective endocarditis during labor. They were kept in a proper up position. Intermittent oxygen and analgesics were provided whenever needed. The second stage of labor was shortened, if necessary, by the use of outlet forceps or vacuum extractor. Oxytocin was used for control of postpartum hemorrhage. Women who had been on anticoagulants were restarted on heparin within four hours of vaginal delivery and eight hours of cesarean delivery. Oral anticoagulants were resumed and heparin discontinued when prothrombin time reached 1.5–2 times normal. The authors recorded maternal and fetal complications such as hemorrhage, thromboembolic complications, and heart failure during the pregnancy, delivery, and puerperium. Neonatal evaluation was completed. It included gestational age, fetal weight, Apgar score, intrapartum complications, thrombosis of the valve. Analysis of thromboembolic complications was 6%.

**Results**

The average age of patients was 25 years in group I, 27.9 years in group II, 26.4 in group III, and 28.8 in group IV. Statistical analysis showed difference between the groups. (Fx was 18.6)

Mitrval mechanical valve was replaced in 27 patients, 18 aortic, and two tricuspid. Thirty-six patients had one replaced valve, 10 had two replaced valves, and one had three.

NYHA class of patients was I and II in majority of cases. In group II and III one patient each had class III, and in group IV, four patients. These 4% of patients came to the hospital with advanced pregnancy. Statistical analysis showed significant difference between the groups (Fx was 3.12). The worst NYHA status was in group IV. Similar data resulted for labor. Nine patients in group IV were in class III NYHA (6%).

The present authors found that 45% of patients required cardiac medications in order to support heart function. The most frequently used medications were digitalis, diuretics, and oral anticoagulants in patients with artificial heart valves. They divided the patients in group IV according to the type of anticoagulation therapy. Group A included 22 patients receiving ethyl biscumacetate during the first 36 weeks of gestation, and intravenous heparin in the last four weeks and after delivery. Group B included 25 patients receiving oral anticoagulant therapy during whole pregnancy. The incidence of hemorrhagic complications during pregnancy was 2.7%, all from subgroup A: one vaginal bleeding, three epistaxis, and one subcutaneous hematoma. After the delivery, the incidence was 4.1%; two patients had hematomas due to episiotomies and four had postpartum hemorrhage, all from subgroup B. One patient had total and one subtotal hysterectomy because of postpartum hemorrhage. In the case of the second patient, who unfortunately died, pathologists found ventricular carcinoma with changes in the liver which completely disturbed coagulation status. Because of small numbers, statistical analysis showed no difference.

One patient from group II had heart failure during pregnancy and six from group IV. After the delivery there was 4% of heart failure: one patient from group II and five patients in group IV. Statistical difference was significant (Fx was 2.5). The majority of heart deteriorations were in patients with artificial heart valves.

When the present authors analyzed factors which influenced the heart failure, they noticed that it occurred in 92% of cases in patients younger than 30 years, which was statistically significant. No significant correlation was found between NYHA class and heart failure which was unexpected. In class I and II, eight patients had heart failure (5.5%), and five patients were in class III (3.4%). Type of operation also influenced the heart failure. In 92% of cases patients had mechanical heart valves (Fx was 7.7).

Number of preterm labors differs statistically between the groups. In group I it was 25% of premature labors, in group II it was 11%, in group III is was 6%, and 23% in group IV (Fx was 2.72). The highest rate was in groups I and IV.

When the present authors analyzed the mode of delivery they noticed that 80% of deliveries were vaginal and only 8% were induced, while others were spontaneous. Cesarean sections were performed in 20% of cases. There was no statistical difference between the groups (Fx was 2). In group I, 75% of patients were delivered by cesarean section, in 18% in group II, 20% in group III, and the least percentage was 6% in group IV. The incidence of forceps was 14% and vacuum application 14%.

Four patients had thromboembolic events before the pregnancy: two had cerebrovascular attacks, and two thrombosis of the valve. Analysis of thromboembolic complications during puerperium showed that one patient had pulmonary and two had cerebral embolisms, all in subgroup A. The incidence of postpartum thromboembolic complications was 6%.

Maternal mortality rate was 2%. Two patients died due to heart failure at three and seven days after the vaginal delivery in group IV. These were uncontrolled patients who came to the hospital just before labor in serious heart conditions, hence postpartum correction was impossible.
The average neonatal birth weight was 3,000 g in group I, 2,600 g in group II, 2,800 g in group III, and 2,600 g in group IV and the difference was statistically significant (Fx was 5.1). APGAR score was less than 7 in 5.4% of cases. Statistical analysis showed influence of maternal NYHA class on Apgar score (Fx was 40.6). One-third of newborns with APGAR less than 8 were from NYHA class II and III. Cesarean section was performed in 60% of these cases. There were two cases of fetal intrauterine death (1.3%), as well as 13 cases of fetal growth restriction (8.9%).

One newborn died of hydrocephalus in group IV subgroup B (0.6%), which can be explained with teratogenic effect of oral anticoagulant therapy when administered in the second trimester causing fetal hemorrhage. One newborn from group II and two from group III died of hypoxic ischemic encephalopathy. Neonatal mortality was 2.7%. In the present study there were no fetuses with congenital heart disease.

Pregnancy in patients with NYHA class I and II after surgical correction can be allowed. Pregnancy places strain on the cardiovascular system and increases risks which are difficult to predict. At the beginning of pregnancy only 6% of patients had unsatisfactory functional status. In group I status did not change during labor and puerperium. In group II one patient changed NYHA class from III to II. In group III, two patients passed from NYHA I to II, and one from NYHA II to III. In group IV, seven patients moved from class I to II and five from class II to III. During delivery functional status changed in 15 patients (10.2%) and majority was with artificial heart valves [14].

Discussion

Four patients suffered thromboembolic events before the pregnancy and three patients during puerperium, all in subgroup A. The incidence of thromboembolic complications was 2%. This signifies that heparin did not prevent thromboembolic complications after delivery. Salazar et al. [2] registered three valve thrombosis and 14 cerebral embolisms in patients who received oral anticoagulant therapy with kumarin, and one patient had cerebral insult under heparin therapy. Ismail et al. [3] found two thromboembolic complications among 76 pregnancies who received heparin therapy.

The incidence of hemorrhagic complications during pregnancy was 2.7% and 4.1% after the delivery, all in subgroup B. The patients who received heparin during the last four weeks and after the delivery rarely suffered from hemorrhagic complications. In comparison Matoras et al. [4] had one postpartum hemorrhage in 59 deliveries of patients with oral anticoagulant therapy. Ismail et al. [3] had seven postpartum hemorrhages among 76 patients. Ayhan et al. [5] had 20% and Avila et al. [6] 23% of hemorrhagic complications in their study.

One patient had heart failure during pregnancy in group II and six in group IV. After delivery there was 4% of heart failure: one patient from group II and five patients from group IV. The majority of heart deteriorations were in patients with artificial heart valves. The incidence of heart failure during the pregnancy was 11% and after delivery it was 9%. Matoras et al. [4] had two heart failures among 59 patients. Mazhar and Gul-e-Irmi [7] noticed 7% of heart failure, Malhorta et al. [8] 5.1%, and Sermer et al. [9] had 18%, and the worst prognosis were in patients with previous complications and patients under oral anticoagulant therapy.

Patients with heart disease, because of impaired circulation, have hypoxia of myometrium and tendency to premature labor. The incidence was 13%, but majority of them were in group with artificial heart valves. Bhutta et al. [10] found 7% of premature labors.

The mode of delivery was 80% vaginal and only 8% was induced. Cesarean sections were performed in 20% of cases. Oron et al. [11] reported 39% of inductions in women with heart disease and no increased rate of cesarean sections or maternal and neonatal morbidity.

One newborn had anomaly (hydrocephalus) in group IV subgroup B (0.6%), which was likely to be due to teratogenic effect of oral anticoagulant therapy when administered in the second trimester. One newborn from group II and two from group III died of hypoxic ischemic encephalopathy. Neonatal mortality was 2.7%. In the present study there were no fetuses with congenital heart disease. The authors noticed two cases of fetal intrauterine death (1.3%) and intrauterine growth restriction of 13 fetuses (8.9%). Mazhar et al. [7] had 5% of fetal intrauterine death, Bhutta et al. [10] 2%, and Kaemmerer et al. [12] one fetus. Kaemmerer et al. [11] noticed 5.4% of fetal congenital heart diseases in their study. Lupton et al. [13] found the inheritance rate of 2-20%. Chaupczak et al. [14] also found increasing rate of inheritance, but increasing rate of cesarean sections as well.

Two patients died therefore maternal mortality rate was less than 2%. Mazhar et al. [7] found 7.1% of maternal mortality rate. Avila et al. [6] had lower rate of 2.7%. Bhutta et al. [10] found 20% mortality rate of patients with heart disease who were not operated.

Conclusions

Pregnancy should be planned according to the heart functional status. Oral anticoagulant therapy should be replaced with heparin and continued for the first 12 weeks, and the last four weeks. In the middle of pregnancy oral anticoagulants should be administered. The present authors suggest ethyl biscumacetate because it showed mild teratogenic effect. They should be monitored by the team of cardiologist, surgeon and obstetrician every three to four weeks.

The preferable way of delivery is vaginal. The present study suggests that there is no need for preterm termination of pregnancy if NYHA status allows it. After the delivery 12-24 hours, heparin should be administered and 48-72 hours
with oral anticoagulants. Worsening of functional heart status was detected in 4% of cases after delivery. Patients with mechanical heart valves were under greater risk for thromboembolic and hemorrhagic complications, heart failure, as well as for fetal anomalies compared to other patients who had heart surgery for congenital and acquired heart diseases.

References


Address reprint requests to: S. PLESINAC, M.D. Dr Subotica 8 11 000 Belgrade (Serbia) e-mail: plesinac@hotmail.com
Experience of assisted reproductive technology at King Abdulaziz University Hospital

H.S.O. Abduljabbar, S.T. Djamil, N.N. Sahly, D.S. Sawan, G.S. Ashour, A. Abduljabbar
Department of Obstetrics & Gynecology, Medical College, King Abdulaziz University, Jeddah (Kingdom of Saudi Arabia)

Summary
Aim: To present the authors’ experience with assisted reproductive technology (ART) at King Abdulaziz University Hospital in Jeddah, Saudi Arabia. Materials and Methods: Retrospective analysis of data collected from the charts of 264 women who were undergoing their first cycle of ART between September 2013 and March 2014. All the women were treated with gonadotropin-releasing hormone (GnRH) antagonist protocol. For all patients, the documented data included age, infertility type, cause, and hormone profile. Number of follicles > 10 mm, endometrial thickness, number of oocytes retrieved, number of fertilized ova, and number of embryos produced, as well as the number transferred, day of transfer, cancellation rate, and treatment administered for luteal phase support (oral and vaginal progesterone) treatment type, and outcome were recorded. The data was analyzed using the Statistical Package for the Social Sciences. Results: The authors included women aged 21 to 39 years (mean ± standard deviation, 32.28 ± 5.51). Patients suffered from primary infertility in 69.7% of the cases; approximately 30% of the women had secondary infertility. Eighty of the 264 patients (30.3%) conceived; however, only 56 women (21.2%) had a live birth. The overall cancellation rate in the patients was 12.1%. The following reasons were documented for cases of failure: no oocytes, 16 (6.1%); no sperm, eight (3.0%); and no embryo, eight (3.0%). Conclusion: The success rate of ART at the present institution falls within the range reported in the medical literature. However, further studies should be conducted to investigate the course and outcome of ART in patients who undergo treatment in this institution.

Key words: Assisted reproductive technology; Infertility; Intracytoplasmic sperm injection; In vitro fertilization.

Introduction
An estimated 10% to 15% of couples suffer from infertility, which is reported to affect men and women in approximately equal proportions [1]. However, infertility is increasingly being overcome through advancements in fertility treatment, especially assisted reproductive technology (ART), which has evolved over the last 30 years into a suite of mainstream medical interventions that have ensued in the birth of over 4.3 million babies worldwide [2].

According to the American Society for Reproductive Medicine, ART is defined as treatments and procedures that involve the use of human oocytes and sperm, or embryos, with the aim of achieving a pregnancy [3]. Based on this definition, ART includes in vitro fertilization (IVF) procedure with or without intracytoplasmic sperm injection (ICSI), while techniques such as intrauterine insemination and induction of ovulation using drugs are excluded [3].

In Australia, the number of ART treatment cycles and live births rose steadily until the late 2000s. According to recent estimates, 3.6% of all women who delivered in Australia in 2009 received some form of ART treatment [4]. In the United States, 176,275 ART treatment cycles were performed at 456 reporting clinics in 2012, resulting in 51,294 live births [5].

The Centers for Disease Control and Prevention reported that although ART is still relatively rare when compared to the potential demand, its use has doubled in the past ten years in the United States [5]. A similar increase in the use of ART is reported in developing countries. In India, for example, about 125 ART clinics were functional in the capital city in 2013 [6]. In the Middle East, however, the growth of ART has been hampered by cultural and religious factors [7, 8]. ART only became popular in an ultraconservative country such as Saudi Arabia after the passing of an Islamic decree in 1980 and a statement by the Islamic Fikh Council in 1984 [9, 10]. Subsequently, about 23 ART centers were established in Saudi Arabia between 1986 and 2007 [11].

In Saudi Arabia, there are limited data on the outcome of IVF. Previous studies [12-14] reported the outcome of ART in obese women and the pregnancy rates achieved with the timing of embryo transfer. Unfortunately, both studies conducted in one IVF center of Reproductive Medicine, Department of Obstetrics & Gynecology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. In this study, the authors present their experience with ART at King Abdulaziz University (IVF center) in Jeddah, Saudi Arabia.
Materials and Methods

A retrospective analysis of 264 consecutive patients who were undergoing their first cycle of ART at the In Vitro Fertilization Center of King Abdulaziz University Hospital, Jeddah between September 2013 and March 2014. The Biomedical Ethics Committee of King Abdulaziz University approved the study. Patients were included provided they fulfilled the following criteria:

- they had a diagnosis of infertility, defined as the failure to achieve a clinical pregnancy after ≥ 12 months of regular unprotected intercourse, [15]
- they were undergoing their first cycle of ART,
- they were aged 18-40 years,
- they had an indication for IVF,
- they received the same antagonist protocol.

Exclusion criteria included all women who were undergoing a repeat ART cycle or those who received a different protocol excluded from the study.

Protocol

Controlled ovarian hyperstimulation achieved by using the gonadotropin-releasing hormone antagonist (GnRH) protocol. Subcutaneous gonadotropin 150-300 IU administered on the third day of the women’s cycle based on their age and weight. On day 6, subcutaneous cetrorelix 0.25 IU administered and cycle progression was monitored by folliculometry and measurement of serum estradiol (E2). In the pre-ovulatory phase, when the follicles attained a size of 18 to 22 mm, final oocyte maturation and release was effected by subcutaneous administration of 5,000 to 10,000 IU of human chorionic gonadotropin (hCG).

Under transvaginal ultrasound guidance, oocytes were retrieved 34 to 36 hours post-hCG administration. Fertilization achieved by IVF or ICSI as clinically indicated. Embryo culture performed using a sequential micro-drop system, and embryos transferred into cleavage media on days 1–3. Patients had embryo transfer when they met the criteria for transfer. Progesterone 400 mg pessaries twice daily or oral dydrogesterone ten mg twice daily administered for luteal phase support. A beta-hCG test performed two weeks following embryo transfer. A transvaginal ultrasound performed after four weeks to determine the number of gestational sacs.

In this study, pregnancy was defined as a positive serum beta-hCG test and the presence of a gestational sac detectable by ultrasound or an ectopic pregnancy. Miscarriage was defined as pregnancy loss following ultrasound confirmation of an intrauterine gestational sac. A live birth was defined as pregnancy resulting in a viable infant. Twins counted as one live birth event.

Data collection

For all patients included in this study, the authors documented the age, infertility type and cause, hormone profile, number of follicles, number of embryos, number of embryos transferred, day of transfer, treatment type (IVF or ICSI), and outcome.

Other data recorded included the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Total dose of gonadotropins administered, day of treatment, number of follicles larger than ten mm, endometrial thickness, number of oocytes retrieved, number of embryos produced, as well as the number transferred, day of transfer, number of fertilized ova, cancellation rate, and treatment administered for luteal phase support (oral and vaginal progesterone) were also recorded.

The etiology of infertility was categorized as follows: anovulation, endometriosis, tubal pathology, male factors, and others. Primary infertility was defined as a condition where a woman had never conceived, while secondary infertility was defined as previous conception irrespective of whether the pregnancy resulted in a live birth. All the women used their own oocytes during the procedure.

Statistical analysis

The data analyzed using the Statistical Package for the Social Sciences Descriptive statistics computed for all study variables. Results expressed as frequency (percentage), mean ± standard deviation (SD), and interquartile range. Odds ratio, and 95% confidences interval with statistically significant when p < 0.05.

Results

The authors included 264 patients aged 21 to 39 years (mean ± SD, 32.28 ± 5.51). Women aged 26-35 years comprised the largest proportion of the sample (Table 1). In over half of the cases, the patients had primary infertility; close to 30% of the women had secondary infertility (Table 2). The etiology of infertility ranged from one factor in one partner to several factors in one or both partners: female factors, 40.9 %, male factors, 22.7 %, and both male and female, 36.4 % (Table 2). Male factors contributed to over half of the cases of infertility. In women, ovarian factors contributed to most of the cases of infertility, documented in approximately 24.2% of the cases. Most patients had ICSI.
The mean FSH and LH levels in the sample were 7.9 ± 2.3 mIU/mL and 11.1 ± 2.4 mIU/mL, respectively (Table 3). The total dose of GnRH received by the patients was 2,770.5 IU (mean, 271.6 ± 50.26 IU), administered during an average of 10.2 days. Table 4 shows the number of follicles, oocytes, and embryos, as well as the number of embryos transferred, the day of transfer, and the patients’ average endometrial thickness. Of the 264 patients treated, 80 conceived (30.3%); however, only 56 women had a live birth, giving a pregnancy rate of 21.2% (Table 5). In cases where fertilization was achieved, the luteal phase was maintained with progesterone pessaries in 188 women (71.2%); oral progesterone was utilized in all other cases. The overall cancellation rate in the present patients was 12.1%. The following reasons were documented for cases of failure: no oocytes, 16 (6.1%); no sperm, eight (3.0%); and no embryo, eight (3.0%).

### Table 3. — Follicle-stimulating hormone and luteinizing hormone levels and gonadotropin dose as well as days of treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/mL)</td>
<td>3.0</td>
<td>15.0</td>
<td>7.3</td>
<td>2.33</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>6.0</td>
<td>17.0</td>
<td>11.1</td>
<td>2.37</td>
</tr>
<tr>
<td>Gonadotropin dose (IU)</td>
<td>130.0</td>
<td>300.0</td>
<td>271.6</td>
<td>50.26</td>
</tr>
<tr>
<td>Days</td>
<td>8.0</td>
<td>14.0</td>
<td>10.2</td>
<td>0.92</td>
</tr>
<tr>
<td>Total units</td>
<td>1500.0</td>
<td>4200.0</td>
<td>2770.5</td>
<td>600.13</td>
</tr>
</tbody>
</table>

FSH, follicle-stimulating hormone; LH, luteinizing hormone; SD, standard deviation.

### Table 4. — Number of follicles, oocytes, embryos and embryos transferred, and other characteristics of assisted reproductive technology treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of follicles</td>
<td>1</td>
<td>17</td>
<td>7.10</td>
<td>2.463</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>0</td>
<td>11</td>
<td>3.66</td>
<td>2.646</td>
</tr>
<tr>
<td>No. of embryos</td>
<td>0</td>
<td>8</td>
<td>2.68</td>
<td>1.920</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>1</td>
<td>4</td>
<td>2.47</td>
<td>1.030</td>
</tr>
<tr>
<td>Day of transfer</td>
<td>2</td>
<td>4</td>
<td>2.23</td>
<td>0.461</td>
</tr>
<tr>
<td>Cryopreservation</td>
<td>0</td>
<td>4</td>
<td>0.33</td>
<td>0.878</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>64</td>
<td>120</td>
<td>105.77</td>
<td>10.854</td>
</tr>
</tbody>
</table>

No., number; SD, standard deviation.

### Table 5. — Outcome of assisted reproductive technology.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative pregnancy test</td>
<td>184</td>
<td>69.7</td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>80</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>264</td>
<td>100</td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Spontaneous Abortion</td>
<td>16</td>
<td>6.1</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Normal delivery</td>
<td>56</td>
<td>21.2</td>
</tr>
</tbody>
</table>

### Table 6. — Factors affecting pregnancy rate in IVF patients (odds ratio and 95% confidence interval).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Non-pregnant</th>
<th>Total</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35</td>
<td>62</td>
<td>99</td>
<td>161</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>18</td>
<td>85</td>
<td>103</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td>Procedure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>16</td>
<td>60</td>
<td>76</td>
</tr>
<tr>
<td>ICSI</td>
<td>64</td>
<td>124</td>
<td>188</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>68</td>
<td>116</td>
<td>184</td>
</tr>
<tr>
<td>Secondary</td>
<td>12</td>
<td>68</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td>Cause</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female factor</td>
<td>44</td>
<td>72</td>
<td>116</td>
</tr>
<tr>
<td>Male factor</td>
<td>36</td>
<td>112</td>
<td>148</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td># of follicles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 8</td>
<td>60</td>
<td>152</td>
<td>212</td>
</tr>
<tr>
<td>&gt; 8</td>
<td>20</td>
<td>32</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 100</td>
<td>44</td>
<td>90</td>
<td>134</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>36</td>
<td>94</td>
<td>130</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td># of fertilized oocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4</td>
<td>56</td>
<td>130</td>
<td>186</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>24</td>
<td>54</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td># embryo transfers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 or less</td>
<td>48</td>
<td>112</td>
<td>160</td>
</tr>
<tr>
<td>More than 2</td>
<td>32</td>
<td>72</td>
<td>104</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td>Day of transfer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>60</td>
<td>156</td>
<td>216</td>
</tr>
<tr>
<td>Day 3</td>
<td>20</td>
<td>28</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>13</td>
<td>66</td>
<td>79</td>
</tr>
<tr>
<td>Vaginal</td>
<td>67</td>
<td>118</td>
<td>185</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>184</td>
<td>264</td>
</tr>
</tbody>
</table>

The mean FSH and LH levels in the sample were 7.9 ± 2.3 mIU/mL and 11.1 ± 2.4 mIU/mL, respectively (Table 3). The total dose of GnRH received by the patients was 2,770.5 IU (mean, 271.6 ± 50.26 IU), administered during an average of 10.2 days. Table 4 shows the number of follicles, oocytes, and embryos, as well as the number of embryos transferred, the day of transfer, and the patients’ average endometrial thickness. Of the 264 patients treated, 80 conceived (30.3%); however, only 56 women had a live birth, giving a pregnancy rate of 21.2% (Table 5). In cases where fertilization was achieved, the luteal phase was maintained with progesterone pessaries in 188 women (71.2%); oral progesterone was utilized in all other cases. The overall cancellation rate in the present patients was 12.1%. The following reasons were documented for cases of failure: no oocytes, 16 (6.1%); no sperm, eight (3.0%); and no embryo, eight (3.0%).

Odds ratio and 95% confidence interval were calculated for the important factors, age above and below 35, procedure IVF and ICSI, type primary and secondary. The cause of infertility whether female and male factors, number of follicles less or more than eight follicles, endometrial thickness less or more 100 mm, number of fertilized oocytes less or more than four, number of embryo transfer two or less or more, day of transfer second or third day. Finally luteal phase was supported with vaginal or oral progesterone. Table 6 shows the results of the important factors that are statistically significant.
Discussion

Saudi Arabia is increasingly moving away from the concept of early marriage, and similar to their counterparts in the West, Saudi women are progressively deferring marriage [16]. This delay in the age of first marriage is paralleled by an increase in the age of first pregnancy, [17] which may consequently lead to a rise in the incidence of infertility related to female reproductive aging [8]. On the other hand, childless couples have to confront the deeply personal experience of infertility or miscarriage in the Saudi society, where like in most Arab cultures, family is, and has always been at the center of life [16].

This report shows that the average age of women who underwent ART at the In Vitro Fertilization Center of King Abdulaziz University Hospital was 32.3 ± 5.5 years, which is similar to the mean age of patients who underwent ART in Taiwan (33.2 ± 4.1 years) and Australia (34.4 ± 4.9 years). While it is generally known that younger women achieve higher rates of pregnancy and live births [19, 20], it is plausible that the women in the current study presented after the age 30 years because they suffered from infertility for several years, and they may have attempted other possible treatments of infertility. Such treatments included seeking the help of a Sheikh, following certain diets, or reading the Qu’ran, which are methods that have been cited as popular options for Saudi couples with infertility problems [21]. In a previous study, [22] other authors suggested that women were on average 32 years of age when they underwent ART due to the fact that they suffered from infertility for 4.0 to 4.4 years and incurred an additional 2.5 to 3.0 years of treatment.

The success rate of any ART procedure is < 30% [23]. In the current study, the pregnancy rate was 30.3%, which is slightly higher than the 19.9% reported among obese women in a study conducted in Riyadh, Saudi Arabia, also higher than the 28.6% among morbidly obese women in the same study [14]. The disparity between the present findings and those of Awartani et al., [14] is unclear, as obesity negatively affects implantation, pregnancy, and live birth rates in IVF candidates [14, 24]. Moreover, the present authors cannot make relevant comparisons between their finding and that of Awartani et al., [14] as they did not investigate the body mass index of the patients in this sample. It should also be pointed that the present study and that of Awartani et al. employed dissimilar stimulation protocols. While Awartani et al. used the long follicular pituitary down regulation protocol, this involved the use of a GnRH antagonist.

Although other factors are essential for a successful ART therapy, patient response to ovarian stimulation is a pivotal factor associated with successful clinical pregnancy [25]. Some authors reported that there were no significant differences between the GnRH agonist long protocol and the antagonist protocol in terms of the duration of ovarian stimulation, number of recombinant FSH ampoules administered, number of oocytes retrieved, estradiol and progesterone serum levels, endometrial thickness, and the zygote- and blastocyst-development rate [26]. Nevertheless, the implantation and clinical pregnancy rates are significantly higher in patients who receive the antagonist protocol (10.6% and 30.3%, respectively) as compared with those of patients who receive the agonist protocol (5.3% and 15.8%, respectively) [26].

In this study, women had an average of 7.1 ± 2.46 follicles. A previous study that considered the various sizes of follicles showed that the pregnancy rates were higher in women who had a higher number of follicles [27]. Other authors demonstrated that the number of small antral follicles measuring 2.1 to 4.0 mm was a significant predictor of viable pregnancy confirmed on ultrasound five weeks after embryo transfer, independent of the patients’ age, number of mature oocytes retrieved, fertilization rates, number of cleaved embryos, and the grade of embryos transferred [28]. In the analyses to assess the relationship between follicle number and size and pregnancy outcome, that was not significant with odds ratio of 1.277 95% confidence interval of (0.754 – 2.162) with \( p = 0.219 \). The average endometrial thickness in this study was 105.8 ± 10.85 mm. One study described the relationship between endometrial thickness on the day of hCG administration and pregnancy outcome in women who underwent IVF and embryo transfer at an IVF center in Riyadh. In their study, [13], the authors found a positive correlation between endometrial thickness on the day of hCG injection and pregnancy rate, suggesting that physicians should consider aiming for a thicker endometrium during ART therapy. Previous research also suggested that pregnancy rates were highest for patients with the thickest endometrial lining [29, 30]. However, other authors suggested a deleterious effect of endometrial thickness of ≥ 14 mm on pregnancy rates [13].

Age is known to be a strong predictor for pregnancy in candidates of ART [31] and the present authors provided an indication when the age was less than 35 years to be an important factor for pregnancy. Due to the fact that this study was a retrospective in nature, it was difficult to retrieve complete data in some cases and this may have introduced bias in the present findings. Taken together, the present findings demonstrate that women who undergo ART procedures at this institution have a pregnancy rate that falls within the range reported in the medical literature. However, because multiple factors affect fertilization rate in candidates of ART, further studies are warranted to study the course and outcome of ART in patients who undergo treatment at this institution.

Conclusion

The success rate of ART at the present institution falls within the range reported in the medical literature. How-
ever, further studies should be conducted to investigate the course and outcome of ART in all patients who undergo treatment of infertility with ART at this institution.

References


Follow-up observational study of “bi-ring method” breast surgery for treating hypermastia and mastoptosis

Department of Plastic Surgery, the First Affiliated Hospital of Fujian Medical University, Fuzhou (China)

Summary
Purpose: This study investigated the efficacy and patient satisfaction of “bi-ring method” breast surgery in 46 patients with hypermastia and/or mastoptosis. Materials and Methods: A questionnaire survey, objective data measurements, visual analysis system survey, and various scoring scales were used to qualitatively and quantitatively assess the patients’ indicators before and after surgery. Results: Statistical analysis showed the following: symptoms and signs in patients with macromastia improved significantly; all patients’ breast shapes improved significantly and became more symmetrical and durable; all patients had minor hidden scars; the nipples and areolas had good feeling postoperatively, and there were relatively few mild complications. Conclusions: The patients’ overall satisfaction was high, indicating that the “bi-ring method” of breast plastic surgery could not only improve the breast shape and boast concealed scars but could significantly improve the patients’ signs and symptoms of hypermastia, but the nipples and areolas had good postoperative feeling and there were few complications. Thus, this is a reasonable surgical approach that is worthy of promotion.

Key words: Bi-ring method; Breast plastic surgery; Hypermastia; Mastoptosis; Patients’ satisfaction.

Introduction
The normal female breast is 250-350 g and has a hemispherical shape. The term “hypermastia” is used to describe a breast above the normal range. Mastoptosis is a more common breast deformity in middle-aged women, especially after childbirth and lactation. Hypermastia and mastoptosis affect the breast’s appearance and increase the likelihood of skin diseases such as eczema and scabies occurring in the folds beneath them and can cause discomfort such as pain in the neck, shoulder, back, and arm, numbness or pain in both hands, breast pain, and even headache, and severe deformities can result in psychological issues [1-6]. There are currently numerous corrective surgery methods for treating hypermastia and mastoptosis, including the lateral breast incision, inside breast incision, areola-cycling incision, vertical incision, and liposuction [7-12]. Among the many hypermastia and mastoptosis correction methods, the “bi-ring method” has the advantages of a small incision, less tissue damage, and minimal scarring; thus, it would be easily accepted by patients, especially Oriental women, and it has become the preferred method by many plastic surgeons in the treatment of mild to moderate hypermastia and mastoptosis. In recent years, foreign and domestic plastic surgeons have made a series of improvements to the bi-ring method [13-17] and achieved even better outcomes. Although the preoperative symptoms and surgical methods have been widely recorded in the literature, patients’ postoperative quality of life and satisfaction have rarely been documented. Thus, the authors performed postoperative follow-up observation of patients who underwent the bi-ring method for the treatment of hypermastia and mastoptosis to assess their outcomes and acceptance and explore whether this method was effective for the correction of hypermastia and mastoptosis.

Materials and Methods
Clinical information
A total of 46 patients (92 breasts; age range, 18-53 years; median age, 35 years) were enrolled in this study and treated in the department of plastic surgery of the First Affiliated Hospital of Fujian Medical University between February 2004 and August 2013. Of these patients, 31 suffered from bilateral hypermastia, 15 cases suffered from simple bilateral mastoptosis, and six had never breastfed. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Fujian Medical University. Written informed consent was obtained from all participants.

Eight patients were excluded for refusing to accept the postoperative follow-up, so the data of a total of 38 patients (26 with bilateral hypermastia and 12 with simple mastoptosis) were analyzed. The follow-up period was an average of 15.95 months (range, six months to five years).

Preoperative design
With the patient in the sitting or standing position, the nipple position was designed to be at the horizontal junction of the midclavicular line and the 4th rib, while the distance from the nipple to the midpoint of the sternal notch was 19-21 cm. The inner ring was designed to set the nipple as the center with an inner areola diameter of 3.5 - 4.0 cm. The size of the outer ring varied ac-
Table 1. — The improvement of preoperative and postoperative symptoms in macromastia patients (x ± s).

<table>
<thead>
<tr>
<th>No.</th>
<th>Symptoms and signs</th>
<th>Preoperation</th>
<th>Postoperation</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bra and clothes were not suitable</td>
<td>7.69±11.77</td>
<td>98.08±16.79</td>
<td>93.3±12.40*</td>
</tr>
<tr>
<td>2</td>
<td>Psychological pressure</td>
<td>3.85±9.20</td>
<td>90.38±14.28</td>
<td>86.54±14.54</td>
</tr>
<tr>
<td>3</td>
<td>Pain from the bra shoulder belt</td>
<td>14.42±23.64</td>
<td>98.08±6.79</td>
<td>83.65±23.39*</td>
</tr>
<tr>
<td>4</td>
<td>Inconvenience in the motion</td>
<td>13.46±21.48</td>
<td>95.19±12.2</td>
<td>81.73±21.86</td>
</tr>
<tr>
<td>5</td>
<td>Neck and shoulder pain</td>
<td>43.27±35.75</td>
<td>95.19±12.29</td>
<td>51.92±31.56*</td>
</tr>
<tr>
<td>6</td>
<td>Upper back pain</td>
<td>38.46±38.81</td>
<td>91.35±15.72</td>
<td>52.88±27.68*</td>
</tr>
<tr>
<td>7</td>
<td>Low back pain</td>
<td>39.42±29.3</td>
<td>94.23±12.86</td>
<td>54.81±23.47*</td>
</tr>
<tr>
<td>8</td>
<td>Shoulder pain</td>
<td>35.58±36.18</td>
<td>96.15±11.60</td>
<td>60.58±34.04*</td>
</tr>
<tr>
<td>9</td>
<td>Itching and eczema in the lower folds of breast</td>
<td>40.38±41.28</td>
<td>97.12±8.15</td>
<td>56.73±39.09*</td>
</tr>
<tr>
<td>10</td>
<td>Arm pain</td>
<td>66.35±21.15</td>
<td>100</td>
<td>33.65±21.15*</td>
</tr>
<tr>
<td>11</td>
<td>Pain or numb in both hands</td>
<td>75.00±10.0</td>
<td>100</td>
<td>25.00±10.00*</td>
</tr>
<tr>
<td>12</td>
<td>Distending pain of breast</td>
<td>62.5±35.53</td>
<td>86.27±28.77</td>
<td>5.77±16.00*</td>
</tr>
<tr>
<td>13</td>
<td>Headache</td>
<td>87.50±20.31</td>
<td>92.31±3.03</td>
<td>4.81±12.29*</td>
</tr>
</tbody>
</table>

Note: *p < 0.05 was considered as the statistical significance.

The surgical procedures were as follows. 1) Incision design (formation of the dermic ring or cap): the epidermis between the rings was removed to form the dermic cap. 2) Dissection of the breast skin and gland: the subcutaneous tissues and breast glands were freed and the partial glands of each patient with hypermastia were removed, while five-cm-wide areas of breast tissue of the outer quadrant (4:00 o’clock position in the left breast, 8:00 o’clock position in the right breast) were kept along with the central breast tissues and breast base fascia. For the patients with mastoptosis, a prosthesis could be implanted behind the pectoralis major muscle according to the breast size and mastoptosis degrees, which required surgeon flexibility, but it was normally five to six cm from the lower point to the inframammary fold, while the upper end was designed to be on the upper edge of the areola with a typical vertical diameter of 12-14 cm and a typical left-right diameter of nine to ten cm. Meanwhile, the range of subdermal dissection was indicated from the outer ring to the base of the breast.

Surgical procedures

The surgical procedures were as follows. 1) Incision design (formation of the dermic ring or cap): the epidermis between the rings was removed to form the dermic cap. 2) Dissection of the breast skin and gland: the subcutaneous tissues and breast glands were freed and the partial glands of each patient with hypermastia were removed, while five-cm-wide areas of breast tissue of the outer quadrant (4:00 o’clock position in the left breast, 8:00 o’clock position in the right breast) were kept along with the central breast tissues and breast base fascia. For the patients with mastoptosis, a prosthesis could be implanted behind the pectoralis major muscle to enable joint correction. 3) Breast shaping and determining of the new nipple position: the flaps of the breast tissues were sutured, and the dermic cap edge was then sutured and fixed with the breast peplus or breast base fascia. The operating table was then adjusted to a semi-sitting position so the nipple and areola could be placed at the horizontal junction of the midclavicular line and the 4th rib; the breast was then reshaped and the size, shape, and symmetry were observed. 4) Drainage: after the bleeding stopped, a negative pressure drainage tube was placed within the wound. 5) Incision suture: 5-O prolene sutures were used in dermic purse-string formation toward the outer ring incision; the dermis and skin were interruptedly sutured and then bandaged and fixed for shaping. The stitches were removed seven to ten days later.

Table 2. — Statistical analysis of preoperative and postoperative breast shape.

<table>
<thead>
<tr>
<th></th>
<th>Preoperation</th>
<th>Three months after surgery</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-point of lower breast fold to the nipple</td>
<td>8.31±1.16</td>
<td>6.91±0.75</td>
<td>1.40±0.52*</td>
</tr>
<tr>
<td>Superternal notch to the nipple</td>
<td>28.54±6.15</td>
<td>22.20±1.66</td>
<td>6.34±4.26*</td>
</tr>
<tr>
<td>Between the 2 nipples</td>
<td>21.42±1.53</td>
<td>20.57±1.02</td>
<td>0.66±0.75*</td>
</tr>
<tr>
<td>Over-nipple chest circumference</td>
<td>92.78±7.48</td>
<td>86.49±4.57</td>
<td>6.30±3.32*</td>
</tr>
<tr>
<td>Areola diameter</td>
<td>4.89±1.21</td>
<td>5.00±0.80</td>
<td>0.11±0.60</td>
</tr>
<tr>
<td>VAS scoring</td>
<td>3.51±1.56</td>
<td>7.57±1.07</td>
<td>4.05±1.97*</td>
</tr>
<tr>
<td>Scores of breast morphology</td>
<td>53.46±2.85</td>
<td>89.32±7.28</td>
<td>35.87±7.97*</td>
</tr>
</tbody>
</table>

Note: *p < 0.05 was considered as the statistical significance.

Statistical analysis

The statistical analysis used SPSS 17.0 statistical software. The measurement data were expressed as mean ± SD. Normally distributed data were subjected to t test analysis, while non-normally distributed data were subjected to the Wilcoxon signed rank test. Values of p < 0.05 were considered statistically significant.

Results

Comparison of symptoms and signs

The improvement degrees of postoperative breast symptoms already had the relative quantitation criteria [18, 19]. The Kerrigan 13-symptom questionnaire was performed before and after surgery [1]. On this questionnaire, “0” indicates “always,” “25” indicates “in most conditions,” “50” indicates “occasionally,” “75” indicates “rare,” and “100” indicates “never.”

Four weeks after the surgery, the results were compared with the preoperative assessments: except for “headache,” the various signs and symptoms were significantly alleviated after surgery (p < 0.05, Table 1). Both the percentages and degrees of the symptoms and signs were significantly reduced; the preoperative symptoms’ means were subjected to the paired t-test, and the obtained value was 13.89, indicating statistical significance (p < 0.001).

Comparison of breast shape

The comparison of breast shapes before and after surgery consisted of objective breast data measurement, visual analysis system (VAS) results, and breast shape scores, and the results were as follows.

In the comparison of mean preoperative and postoperative objective measurement data, the “areola diameter” had a value of p > 0.05, but the data of the other groups were significantly different (p < 0.05, Table 2). The follow-up photos showed that the postoperative breast shapes were significantly improved (Figures 1, 2).
Figure 1. — 25-years-old, comparisons of breast shape and scar before and after surgery in the hypermastia case. A, B, C: before the surgery; D, E, F: two years after the surgery, the breast shape was normal and natural and the scar was not obvious.

Figure 2. — 30-years-old, the breast shape and scar before and after surgery in the simple moderate mastoptosis case. A, B, C: before the surgery; D, E, F: six months after the surgery, the breast shape was natural and the scar was not obvious.
were significantly improving as time went on (Figures 1, 2). When the patients were followed up for > one year was high. In the comparison of postoperative objective measurement data with the standard range of the Chinese population, the p values of “distance between the two nipples” and “over-nipple chest circumference” were > 0.05, while the differences between the other values were statistically significant (Table 3) and the postoperative measurement data were all closer to the standard values (Table 2).

**Assessment of postoperative scars**

All of the patients’ postoperative scars were assessed in addition to the subjective self-feeling assessment, for which we used a grade 0-3 scoring sheet and the Vancouver Scar Scoring Scale for the qualitative and quantitative assessments. The degrees of scar improvement six months after the surgery are described below.

Within three postoperative months, the nipple-centered fold was visible, but it disappeared three to six months after the surgery; by six months after the surgery, most appeared as a small scar without obvious hyperplasia, and most were light red or white, not higher than the skin surface, soft, and did not itch. Four patients had visible areola scar hyperplasia one year after the surgery, but the overall satisfaction toward the scars of both those and the other patients who participated in the pre- and postoperative surveys. The same approaches were used to solve the pre- and postoperative problems, and the same scoring systems were used at two time points. The patients’ individual differences were minimized through the use of a standardized grading system.

This study’s results were similar with those reported in earlier studies. Birtchnell et al. and Goulart et al. [22, 23] found that the motivation of patients with hypermastia to seek surgery was mainly to ease the extreme physical discomfort or intense psychological distress caused by the hy-

**Assessment of postoperative nipple sensation and lactation function**

The grade 0-3 scoring method was used to rate the sensations of pain, temperature, and feeling of the areola and nipple. A score of “0” represented no feeling, “1” represented a serious decline versus preoperative degree, “2” represented dysesthesia compared to preoperative degree, and “3” represented the same degree as that observed prior to surgery. The six-month follow-up exam revealed no cases of sensation loss or severe decline, and the only case of postoperative complications expressed that the postoperative nipple sensation was slower than the preoperative (Table 5). After the long-term follow-up, the six patients who had not previously breastfed were able to lactate normally.

**Evaluation of postoperative complications**

A total of four patients demonstrated postoperative complications: three suffered from wound dehiscence upon stitches removal, but healed after a general dressing was applied, while one patient with severe mastoptosis exhibited a poor blood supply to the right nipple and areola in the early postoperative stage. In this patient, partial areolar flap necrosis appeared, but the wound healed after being dressed; three years later, the breast shape was natural, but the scar on the right breast was obvious, the areolar diameter was slightly larger than that of the contralateral breast, and the feeling in the nipple and areola was dysesthetic.

**Discussion**

Having full, round, and moderately sized breasts is an important indicator of female physical beauty. Hypermastia and mastoptosis not only negatively impact female appearance but also trigger a series of uncomfortable physical and psychological symptoms. The bi-ring method of breast plastic surgery not only improves the symptoms of patients with hypermastia but also improves their physical appearance; because of the concealed scar and good postoperative nipple and areola sensation, it has become the ideal surgical method for the treatment of mild to moderate hypermastia and mastoptosis. It has also been used in recent years in breast-conserving surgery for breast cancer due to wide patient acceptance of the resulting concealed scar [20, 21].

The method of this follow-up study was simple, and some surveys could be performed by telephone; thus, most patients participated in the pre- and postoperative surveys. The same approaches were used to solve the pre- and postoperative problems, and the same scoring systems were used at two time points. The patients’ individual differences were minimized through the use of a standardized grading system.
permastia, whereas only a few patients came to the clinic for aesthetic purposes. This study showed that in the rest-
ing state, the surgery significantly alleviated the signs and symptoms of the patients with hypermastia.

During this follow-up, the preoperative scores of patients with hypermastia and mastoptosis, either through the VAS self-evaluation or the breast shape scoring sheet, showed relatively low scores, while the postoperative scores were significantly improved and the differences were significant compared to the preoperative scores; thus, the patients’ self-evaluations were very similar to the physicians’ evaluations of the breast shape improvements.

Comparison of objective measurement data before and after surgery suggested that through the surgery, the breast shapes were changed, the positions of the nipple and areola were improved and corrected, and the areolar diameter of the patients with mastoptosis was increased compared to the preoperative measurements, which might be due to the postoperative incision tension and scar hyperplasia, while that of the patients with hypermastia was reduced compared to the preoperative values, probably because of gravity and other reasons that made the preoperative diameters larger than those of other women. After the surgery, the breast tissues were significantly reduced and the effect of gravity was reduced, which improved the areolar diameter. The postoperative indexes were closer to the average standard range of Chinese women; namely, the postoperative breast shape was much closer to the perfect standard breast shape.

Gasperoni et al. [24] thought that the correct preoperative evaluation and design were the keys to the good results of glandular shaping in reductive mammoplasty. During the bi-ring method design, an overly small inner ring would increase the skin folds around the areola after suturing, while an overly large inner ring would impact the overall appearance of the areola and breast, while an overly large gap between the inner and outer rings would result in too much incision tension and the incision scar would be correspondingly much more apparent. The scar of this surgical incision was located around the areola and relatively concealed, which avoided the vertical scar that results in a traditional operation; the postoperative VAS scores and the Vancouver Scar Scoring sheet showed that the scars were slight and the patients’ overall satisfaction was high. The proliferation of the scar was related with both the local tension and the patient’s body characteristic, and this point of view has already been confirmed in many studies.

The sensations of the nipple and areola participate in sexual excitation, and mainly accepted the govern of anterior and lateral cutaneous branches of intercostal nerves, while the strikes of these nerve branches within the breast parenchyma were still lack of the consistent reports [25-27]. However, the lateral cutaneous branch of the 4th intercostal nerve has been recognized as the most important control nerve, so when the mammary glands were simply set as the pedicle, it should be noted that this lateral pedicle should be retained so it could maximally retain the lateral cutaneous branch of the 4th intercostal nerve. Therefore, the surgical method main retained the five-cm-wide breast tissues of the outer quadrant (4:00 o’clock position in the left breast, 8:00 o’clock position in the right breast) so that the blood supply and sensation function of the nipple and areola could be retained. Through the follow-up, the sensations of nipple and areola were well preserved and basically did not differ from preoperative values. Compared with the various glands dermic pedicle technologies, the bi-ring method could minimize the injuries from the central pedicle toward the breast duct, thus maximizing the lactation function.

This study showed that the bi-ring method could guarantee minor and concealed scars; the sensation functions of the nipple and areola resulted in good recovery; postoperative complication rates were rare and minor; and the operation was simple, safe and reliable, so it could be an ideal surgical method.

Nowadays, various surgical procedures have their own shortcomings and deficiencies, which still require effort from cosmetic plastic surgeons, and studies of good recovery of breast functions are still important.

Acknowledgements

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References


Address reprint requests to:
B. WANG, M.D.
Department of Plastic Surgery
The first Affiliated Hospital of Fujian Medical University
Fuzhou 350005 (China)
e-mail: cnbiaowang@126.com
Fetal ventriculomegaly during pregnancy course, outcome, and psychomotor development of born children

J. Dukanac Stamenkovic1,2, M. Steric1, L. Srbinovic1, T. Janjic1, S. Vrzić Petronijevic1,2, M. Petronijevic1,2, A. Cetkovic1

1 Clinic for Gynecology and Obstetrics, Clinical Center of Serbia; 2 Faculty of Medicine, University of Belgrade
3 Clinic for Gynecology and Obstetrics, Narodni front Belgrade, Belgrade (Serbia)

Introduction

Malformations of the central nervous system are the most complicated and the most common congenital disorder with a prevalence of one in 100 to one in 500 newborns. Regular ultrasound examinations are the most important aspect of detecting these anomalies [1]. The etiology of the disease is variable and can be genetic (chromosomal abnormalities), secondary (as a consequence of intracranial hemorrhage, hypoxic-ischemic lesions, transplacental infection, obstruction of the flow of cerebrospinal fluid) or multifactorial [2, 3].

Ventriculomegaly is defined as an increase in the ventricular size regardless of the degree and etiology of enlargement. Hydrocephalus refers to a heavier degree of ventriculomegaly. Transverse diameter (width) of atrium measurement is the most widely accepted method for ultrasound evaluation of the size of the lateral ventricles of the brain. It is measured at the level of choroid plexus glomus. Ventriculomegaly is divided according to the degree of enlargement in mild (10-12 mm), moderate (13-15 mm), and severe (> 15 mm). The term ‘borderline’ includes mild and moderate ventriculomegaly and represent ‘gray zone’, because the authors do not agree that it is real ventriculomegaly or just a phase in the normal development of the cerebral ventricles [4-6]. In the case of diagnosis of borderline ventriculomegaly, sometimes it is difficult to make a final decision on the further course of pregnancy and advise parents. Mild ventriculomegaly frequently affects both lateral ventricles, and it can also be unilateral or asymmetric [7]. Isolated ventriculomegaly is defined as a ventricular enlargement with normal intraventricular pressure, which is not associated with other sonographic abnormalities of the central nervous system and accounts for 20% of sonographically detected fetal ventriculomegaly. It is considered a dynamic process, so that from the moment of diagnosis until the end of pregnancy may cause spontaneous regression, but also can progress to a severe form [8, 9].

The objectives of this study were as follows: to present the course and outcome of pregnancies complicated with fetal ventriculomegaly, determine the association between prenatal ultrasound diagnoses and definitive postnatal diagnosis or diagnoses after autopsy and additional analysis, and to monitor the psychomotor development of children born with ventriculomegaly.

Materials and Methods

Time and place of study implementation

The research was conducted at the Department of Gynecology and Obstetrics, Clinical center of Serbia in the period from January 2002 until December 2012.

Respondents - monitoring units

The study included 62 pregnant women who were attending a regular ultrasound examinations at the Department of Gynecology and Obstetrics, Clinical Center of Serbia, or patients who were referred from other institutions in Serbia.

Results

Ventriculomegalies were divided into three groups: mild, moderate, and severe or hydrocephalus. The most common were severe ventriculomegalies, with 34 cases (55%). Of all pregnancies complicated with ventriculomegalies, 61% were terminated. Among those continued, 88% had normal psychomotor development. In 97% ultrasonographic diagnosis was confirmed.

Conclusion

Majority of pregnancies complicated with ventriculomegaly were continued and most of the children born with anomalies had normal psychomotor development.

Key words: Ventriculomegaly; Fetus; Psychomotor development; Outcome.
ferred from other institutions in Serbia. The criteria for inclusion in this study were visualized ventriculomegaly by ultrasound, insight into the histopathological diagnosis if the pregnancy was terminated, and telephone contact with parents in order to assess the psychomotor development of children. Criteria for exclusion from the study were the inability to establish phone contact with parents and non-disclosure in the histopathologic diagnosis. Five patients were excluded from the study, whereas the present authors were not able to perform phone contact with four respondents, and in one of the interviewees did not have access to histopathologic diagnosis.

Clinical methodology

If during a routine ultrasound examination there was suspected ventriculomegaly, it was advisable to review patient by a multidisciplinary Consilium for fetal anomalies consisted of the perinatologist, child neurologist, neurosurgeon, and geneticist who followed the further course of pregnancy. In addition to the ultrasound examination, some examinees were advised additional diagnostic methods and analysis to determine the precise diagnosis and etiology of diseases, such as screening for infections toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus (TORCH), karyotyping, and magnetic resonance imaging (MRI).

Statistical methodology

The survey was designed as a retrospective study. Information during pregnancy and their outcomes were collected from Consilium for fetal anomalies reports, fetal autopsy reports, and by telephone contact to obtain information on postnatal psychomotor development of children. Observation characteristics that the present authors followed were: maternal age, parity history, history of similar or other congenital anomalies in a family or in previous pregnancies, weeks of gestation in which women were first shown Consilium for fetal anomalies, the total number of consultative examination, karyotype, MRI findings, mothers' comorbidities, prenatal sonoFigureic diagnosis of abnormalities, the diagnosis at autopsy or postnatal diagnosis after birth, institution where birth took place, way of delivery (vaginal delivery or cesarean section), child's sex, and psychomotor development.

In order to determine the correlation between prenatal and final diagnosis, the authors divided them into three groups. In group 1 were pregnancies where the prenatal diagnosis and the final diagnosis after birth or autopsy fully matched, regardless of whether they were isolated or associated anomalies. Group 2 included pregnancies in which diagnosis at birth or after an autopsy confirmed prenatal diagnosis, but cerebral and extracerebral anomalies were discovered that were not seen by ultrasound. Group 3 classified anomalies that prenatally could not be diagnosed, so the diagnosis was made after the birth or autopsy or wrongly diagnosed malformations.

Statistical analysis included descriptive statistics (integers, percentages of proportion, and mean, standard deviation). The results are presented Figureically and in tabular form.

Results

Ventriculomegalies were divided into three groups: mild, moderate, and severe or hydrocephalus. Total number of all ventriculomegalies in the present study were 62. The most common were severe ventriculomegalies, with 34 cases (55%), as shown in Figure 1.

More than half of women whose pregnancies were complicated with ventriculomegaly were nulliparous (Table 1).

Figure 1. — Ventriculomegaly classification according to severity of ventricle dilatation.

Results

Almost 80% of the examinees had one medical consultations, while only five of them had more than two serial consultative examinations (Table 2).

All of the diagnosed mild ventriculomegalies were isolated (Table 3). Mean gestational week when the patient first presented to Consilium for fetal anomalies was 32 (ranging from 28th to 37th week). The average age of mothers whose pregnancies complicated with mild ventriculomegaly was 30.6 years (range 20 - 39). None of the multiparous, previous pregnancies were complicated with central nervous sys-
tem anomalies. All pregnancies complicated with mild ventriculomegaly were monofetal. In four (29%) fetuses, karyotyping was done and all of them were normal and in three (21%) of respondents underwent TORCH infection screening and in all it was negative. Of additional diagnostic methods in order to confirm the diagnosis, MRI was performed in six (36%), in three of them MRI coincided with the sonoFigureic findings, and for two patients data was not available. As for the outcome of pregnancy, all the children were live births and all were born in the Department of Obstetrics and Gynecology, Clinical centre of Serbia. Four of them (16.7%) gave birth in secondary level institutions, and the remaining ten (88.3%) in the Department of Obstetrics and Gynecology, Clinical centre of Serbia. Four pregnancies were completed at term, and all children were eutrophic. In one infant mild ventriculomegaly by the end of the first year of life progressed to hydrocephalus, and set a new diagnosis: cerebral gigantism, in which a child has epilepsy by type of grand mal. At the time the present study was conducted, the child was less than three-years-old (follow-up period 35 months). Karyotype of this child showed inversion of fifth chromosome that occurred de novo. In the remaining 11 (92%), postnatal sonoFigureic findings and psychomotor development were normal (Table 4). The average follow up of psychomotor development of children was 30 months (range six to 50). One was a twin pregnancy as a result of in vitro fertilization, in which only one fetus was diagnosed with moderate ventriculomegaly, while the other fetus was without any abnormality. Other pregnancies were monofetal. One pregnancy was complicated by mother’s gestational diabetes. In four (33%) fetal karyotype was made, which was normal in three, and in one the result was unknown. In seven (58%) of them MRI was performed and were matched with the sonoFigureic findings. During one pregnancy screening was made for TORCH infections, which was negative. The largest number of moderate ventriculomegaly was isolated, with as many as 13 fetuses (92.8%). In only one fetus (7.2%), ventriculomegaly was as-isolated, with as many as 13 fetuses (92.8%). In only one fetus (7.2%), ventriculomegaly was associated with ascites (Table 3).

The average age of women whose pregnancies were complicated with moderate ventriculomegaly was 30.1 years (range 18-39). The patient was first examined by Consilium for fetal anomalies average at 30th gestational week (range from 26th to 37th). No multiparous during previous pregnancy had central nervous system anomalies. In two patients (14.3%), pregnancy was terminated. One pregnancy was terminated at 36th week. During pregnancy MRI was performed, which showed that in addition to ventriculomegaly there was also porencephalic cyst of severe degree. Screening for TORCH infections was negative. Karyotyping was also performed, but the authors had no information on the results. An autopsy revealed ventriculomegaly of severe degree, so that it was placed into Group 2 (Figure 2). The second pregnancy was terminated at 30th gestational week, the karyotype was normal, and MRI findings was unknown; TORCH infections screening was negative. An autopsy confirmed moderate ventriculomegaly, which placed it in Group 1 (Figure 2).

Of the 12 (85.7%) women who continued the pregnancy, two of them (16.7%) gave birth in secondary level institutions, and the remaining ten (88.3%) in the Department of Obstetrics and Gynecology, Clinical centre of Serbia. Four (33%) pregnancies were ended by cesarean section, and the remaining eight (67%) delivered by the vaginal route. All pregnancies were completed at term, and all children were eutrophic. In one infant mild ventriculomegaly by the end of the first year of life progressed to hydrocephalus, and set a new diagnosis: cerebral gigantism, in which a child has epilepsy by type of grand mal. At the time the present study was conducted, the child was less than three-years-old (follow-up period 35 months). Karyotype of this child showed inversion of fifth chromosome that occurred de novo. In the remaining 11 (92%), postnatal sonoFigureic findings and psychomotor development were normal (Table 4). The average follow up of psychomotor development of children was 30 months (range six to 50). One was a twin pregnancy as a result of in vitro fertilization, in which only one fetus was diagnosed with moderate ventriculomegaly, while the other fetus was without any abnormality. Other pregnancies were monofetal. One pregnancy was complicated by mother’s gestational diabetes. In four (33%) fetal karyotype was made, which was normal in three, and in one the result was unknown. In seven (58%) of them MRI was performed and were matched with the sonoFigureic findings. During one pregnancy screening was made for TORCH infections, which was negative. The largest number of moderate ventriculomegaly was isolated, with as many as 13 fetuses (92.8%). In only one fetus (7.2%), ventriculomegaly was associated with ascites (Table 3).

The average age of women whose pregnancies were complicated with hydrocephalus was 29.7 years (range 19-42). The fetuses were first shown to the Consilium for fetal anomalies at an average at 30th gestational week (range of 17th to 37th), with four (11.8%) first presented before the
24th week of gestation, and all of the pregnancies were terminated. In three patients (11%) in which the current pregnancies were complicated by fetal hydrocephalus and terminated, the previous pregnancies were also diagnosed with fetal hydrocephalus; a karyotype was performed in only one of them, where the authors had no information on the outcome. In two patients (7%), whose current pregnancy was terminated, outcome of previous pregnancies was infant death in the first seven days of life, the causes unknown. The largest number of pregnancies complicated by severe ventriculomegaly fetuses (79% or 27 pregnancies) were terminated (Table 4).

The patients whose pregnancies were complicated by severe ventriculomegaly of fetus and continued are shown in Table 5. The average age of mothers who continued pregnancy was 31 years (range 19-38); in only one patient the previous pregnancy was also complicated by hydrocephalus. In these patients karyotyping was not performed although it was suggested. All other respondents, six of them were nulliparous. Mean gestational weeks in which these respondents were shown to Consilium staff team was 33.4 weeks (range 31 to 37). All the patients had one council. Karyotype was performed in three fetuses (43%) and each of them was normal. Two patients (29%) underwent MRI, which coincided with the ultrasound findings. None of the patients during pregnancy had undergone TORCH screening. All pregnancies were monofetal.

According Table 5 we can see that the prenatal and postnatal findings are fully matched in almost all cases examined (6-86%). In one case, No. 3 in Table 5, prenatal ultrasound diagnosed anechogenous formation of posterior fossa diameter 70 x 40 mm, with next consultative examinations showed progressive growth, but it was not possible to set a definitive diagnosis. Postnatally, it was found that it was the hydrocephalus caused by arterior of Sylvian aqueduct, so the authors placed it in Group 3. In two cases, numbering 2 and 3 the cause was known, while in others the cause remains unknown. All severe ventriculomegaly were isolated, without associated cerebral or extracerebral anomalies (Table 3). One of the fetuses (14%) was delivered by cesarean section; the other six (86%) vaginally. Only one patient was delivered at the secondary level institution, while all the others were delivered at the Department of Gynecology and Obstetrics. One infant was born prematurely at 33rd gestational week, three at the 37th week, two at 38th, and one in 39th week of gestation. The average psychomotor development follow-up of children born alive was more then three years, more precisely 39.7 months (range 33-58). Only one child died in the neonatal period after seven days (number 3). Three children had a neurosurgical operation (ventriculo-peritoneal shunt application) and all at the time of the study had normal psychomotor development. Two children had two operations, where one had normal psychomotor development, while the second, which was prematurely born, had problems with talking, does not walk, and has trouble seeing. One child in whom the diagnosis was obstructive hydrocephalus, had five surgeries, had epilepsy, and spastic hemiparesis.

<table>
<thead>
<tr>
<th>Table 4. — Outcomes of pregnancies complicated with ventriculomegaly.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number (%)</strong></td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Ventriculomegaly</td>
</tr>
<tr>
<td>- Mild</td>
</tr>
<tr>
<td>- Moderate</td>
</tr>
<tr>
<td>- Severe</td>
</tr>
</tbody>
</table>

Mild VM refers to seizures by type of grand mal, one ‘-’ in case of severe VM, epilepsy, and spastic hemiparesis, the other ‘-’ in case of severe VM: do not walk, blind, and speaking issues.

<table>
<thead>
<tr>
<th>Table 5. — Pregnancies complicated with severe ventriculomegaly and continued.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Karyotype</strong></td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1. Not done</td>
</tr>
<tr>
<td>2. Not done</td>
</tr>
<tr>
<td>3. Not done</td>
</tr>
<tr>
<td>4. Normal</td>
</tr>
<tr>
<td>5. Normal</td>
</tr>
<tr>
<td>6. Not done</td>
</tr>
<tr>
<td>7. Normal</td>
</tr>
</tbody>
</table>

* vag-vaginal delivery; * sc-cesarean section.
The average age of women who terminated their pregnancies complicated with severe ventriculomegaly of fetus was 39.7 years (range 21-42). They were first presented to the Consilium at an average of 29th week (range from 17th to 37th). Most of the patients, 21 (78%) were presented only once, after which the final decision on the further course of pregnancy was made, while four of them (15%) were at two consultations and one women (7%) had four consulting examinations. Mean gestational weeks in which the abortion was performed was at 30th weeks (range from 17th to 38th). Karyotyping was performed in seven (26%) women who terminated their pregnancies and for all it was normal. MRI was performed in 11 (41%) and findings coincided with the sonoFigureic findings. In two patients (7%) histopathologic analysis of the placenta revealed the presence of chronic transplacental infection. One of these histological images was compatible with toxoplasmosis, a screening for TORCH infections was positive (IgM and IgG antibodies to toxoplasmosis were increased), while the others beside the placental infection, was present also infections of the brain, lungs, and kidneys, with unknown infectious agent, TORCH screening was not done, and hydrocephalus was a combination of internal and external. In two patients (7%) in the histology, brain tissue revealed the presence of reactive gliosis and existence siderophages indicating possible vascular etiology of severe ventriculomegaly. Based on the autopsy and histopathological analysis, nine fetuses (26%) were diagnosed with internal hydrocephalus, suggesting obstructive etiology at level of interventricular openings or Sylvian aqueduct, as shown in Figure 3.

Three women (11%) had comorbidities that also complicated pregnancy: one patient had hypertension, the second one systemic lupus erythematosus, and the third one myxoma of cardiac chambers, deep vein thrombosis, and aneurysm of interatrial septum. Three pregnancies were twin pregnancies (11%), with the finding of the central nervous system, with the other fetuses were normal. In 24 examinees (89%), fetal prenatal and postnatal diagnoses were completely matched, while the three fetuses (11%), postnatally at autopsy revealed additional extracerebral anomalies (Table 3). In two fetuses there was hepatomegaly, and in one facial dysmorphia with splenomegaly and ascites in whom the known causative infectious agent was toxoplasmosis. In two more fetuses there was the existence of associated extracerebral anomalies diagnosed prenatally and postnatally confirmed: in one, cystic kidney tumor, and in the second ventricular septal defect. As for fetal sex, the authors had data for only ten of the fetuses, in which six were female and were four males.

Overall, if we exclude all pregnancies complicated with mild and moderate ventriculomegaly which were not terminated, the largest number, 31 of the 36 fetuses (86%) with ventriculomegaly are classified in Group 1. In Group 2 there were four of them (11%), while in Group 3 there was only one fetus (3%), as shown in Figure 3.

In Table 4 we can see that more than half of pregnancies complicated with ventriculomegaly, 53%, were terminated. In 61% of examinees confirmed the presence of anomalies, with the largest number of confirmed anomalies was among severe ventriculomegaly. In 88% of cases where the pregnancy continued, psychomotor development was normal.

Discussion

The incidence of chromosomal abnormalities present in fetuses diagnosed with initially mild ventriculomegaly according to literature is about 4% [10-12]. According to published studies, the majority of fetuses with mild ventriculomegaly and abnormal karyotype have associated anomalies. In case of anomalies associated with mild ventriculomegaly, the incidence of aneuploidy (usually trisomy of chromosome 21q and 18q) was 2-3%, 14.2% with moderate ventriculomegaly, and 17.4% with severe ventriculomegaly [13, 14]. In the present study, pathological karyogram showed only one fetus (8%) with moderate ventriculomegaly which was isolated. The present study showed in one fetal karyotype (5%) with severe ventriculomegaly that there was hereditary pericentric inversion of chromosome, which probably does not have clinical significance.

Gaglioti et al. followed children with prenatally diagnosed ventriculomegaly during a period of two years and demonstrated that 97.7% of children with mild ventriculomegaly survive, 80% with moderate, and 33.3% with severe ventriculomegaly. Of those who survive, normal psychomotor development is present in 93% of cases with mild, 75% with moderate, and 62.5% with severe ventriculomegaly [15]. An incidence of delayed psychomotor development in the case of isolated mild ventriculomegaly in the literature ranges between 0% and 36%, with approximately 90% of all investigated cases that have normal psychomotor development [9]. Sherif et al. have shown that the risk of delayed psychomotor development is significantly higher if the width of ven-
The atrial septum is more than 12 mm [5]. According to a
British author, the average incidence of abnormal psy-
chomotor development in the literature ranges between 7% and 46%. British researchers also examined perinatal out-
come in fetuses with severe ventriculomegaly where this anomaly was diagnosed if the width of the atrium was larger than 15 mm. Half of the pregnancies were terminated, and the other half were live-born children. Of all live births 20% died within four months, and of the remaining cases only 13% had normal psychomotor development. As for preg-
nancies that are continued, only 10% of respondents at the time of research, at the age of one year of life had normal psychomotor development. The average follow up was 15 months. Twenty percent died in the neonatal period, and 20% underwent neurosurgical intervention (ventriculo-peritoneal shunt), and both children had cerebral palsy [8]. In the pres-
ent study, all 14 fetuses with prenatally diagnosed mild ven-
triculomegaly after birth had normal findings of central
nervous system at the time of the research normal psy-
chomotor development, with average follow-up period 32
months (range 7-42). As for moderate ventriculomegaly, 12
pregnancies out of 14 (86%) continued. As the present au-
thors have already mentioned in one case (8%), moderate
ventriculomegaly progressed to severe ventriculomegaly,
while in the remaining 11 (92%) postnatal sonoFigureic find-
ings and psychomotor development were normal. The aver-
age follow up of psychomotor development was 30 months (range 6-50). The present study included 34 pregnancies complicated with severe ventriculomegaly, of which only seven continued (20.5%). In the present research, six chil-
dren were alive (85.7%), of whom four had normal psy-
chomotor development (66.7%), vision, walking and talking
issues, and another has epilepsy and spastic hemiparesis. One child died after seven days of life (14.3%). Pier et al. con-
cluded that in approximately 29% of cases of sonoFigureically diagnosed isolated ventriculomegaly leads to spontaneous regression, in 57% of cases it remains constant, and in 14% of cases ventriculomegaly progresses [16]. Gold-
stein et al. performed a systematic review of the literature which covered 577 patients with moderate ventriculomegaly. Normal psychomotor development was seen in 85.2% of cases, slightly marred by 7.8%, while severe mental retarda-
tion was present in 7%. Given the fact that these authors fol-
lowed psychomotor development in two time periods, they concluded that the percentage of those who had normal psy-
chomotor development was lower after a longer follow-up period, which shows the importance of studies of children with anomalies of the central nervous system, accompanied by longer period of time are required in order to obtain more precise information on the prognosis of the disease [17]. Ouahba et al. conducted a prospective study, involving 101 newborns with isolated mild and moderate ventriculomegaly (ventricle width to 15 mm). The mean length of follow-up was 43.81 months (range 2-127). Normal psychomotor de-
velopment was registered in 88% of patients, while the re-
maining 12% had neurological disease or some degree of
psychomotor retardation. There was no difference in the out-
come between unilateral and bilateral ventriculomegaly [12].

In a study of French researchers in a group of severe ven-
triculomegaly, 90% of pregnancies were terminated, and
10% of pregnancies were continued, as was the case with only one patient whose child had normal psychomotor de-
velopment. In the group of mild to moderate ventricu-
losegaly, 32% of pregnancies were terminated. Of
pregnancies that are continued, 91% of infants had normal
psychomotor development, and the remaining 9% had a
handicap (hemiplegia and epilepsy) [15]. In the present
study, all women with mild ventriculomegaly of fetus con-
tinued pregnancy, two (14.3%) with moderate ventricu-
losegaly had terminated their pregnancy, while the group of
severe ventriculomegaly had the highest percentage of abor-
tions amounting to 79.4%. In the group of isolated ventricu-
losegaly in an Irish study, only one pregnancy (5%) was
terminated, 26.3% were stillborn, 10.5% died in the neon-
al period, while more than half (52.6%) were liveborn. Am-
ong live births, only one child (10%) had normal psy-
chomotor development at the time of the survey with four
years of age, 40% mildly retarded psychomotor develop-
ment, while 50% were profoundly disabled, including one
child with agenesis of corpus callosum body with vision is-
sues [13].

The present authors can conclude since the ventricu-
losegaly is the most common central nervous system
anomaly, therefore particular attention must be paid when
diagnosing dilated ventricles atria, which should include a
thoroughly review of the anatomy of the fetus. Given the
fact that Group 3 was 3% of the cases, the authors conclude
that ultrasound is a reliable diagnostic tool in the diagnosis of
central nervous system anomalies. It is necessary to con-
duct more follow-up studies with longer research periods,
in order to make conclusions on psychomotor development of
children with brain abnormalities.

References


Address reprint requests to:
M. STERIC, M.D.
Bulevar kralja Aleksandra 149/10
Belgrade (Serbia)
e-mail: steric.milena@gmail.com
Correlation of twisting motion phase and infantile spasms in high risk infants

Y.Q. Wang1,2, Z.X. Yang2, P. Zhu3, G.X. Gu1
1 Department of Child Health, Affiliated Childrens’ Hospital of Soochow University, Suzhou
2 Rehabilitation Department, Xuzhou Children’s Hospital, Xuzhou (China)

Summary
Objective: The aim of this study was to investigate the correlation of twisting motion phase and infantile spasms in high risk infants. Materials and Methods: One hundred seventy-eight high-risk newborns experiencing follow-up in the rehabilitation phase were selected and full-body motion quality assessment was performed in the twisting motion phase. The occurrence of infants with infantile spasms after 12 months (corrected age) was statistically analyzed. Results: No clear correlation was found between monotonous movement twisting motion phase and infantile spasms, and spasm synchronized movement had no definite prediction for infantile spasms. The incidence of infant spasm with movement form having spastic synchronized characteristics had significant difference compared with monotonous systemic movement (p < 0.01). The sensitivity of predictive rate for spasm-synchronous movement of infantile spasms was 90.9%, the specificity was 96.8%, the positive predictive value was 80%, and the negative predictive value was 98.7%. Conclusions: Spasm synchronized movement had some predictive value for infantile spasms in twisting motion stage. The newborns with this kind of movement form should be checked by regularly ambulatory EEG.

Key words: General movements; Twisting motion; Infantile spasms; Risk newborns.

Introduction
Infantile spasms is a special epilepsy, also known as West syndrome, which was firstly described in detail by British doctors West in 1841 with three characteristics (nod seizures, progressive mental regression, and high-degree imperfect EEG) [1]. The disease is the most common form of age-dependent epileptic encephalopathy with characteristics such as difficult to be treated and poor prognosis. Mental development retardation is often found in the follow-up of infants with infantile spasms, and many merged infantile spasms also existed in infants with cerebral palsy or mental retardation. The average incidence of the disease is about 0.31% [2]. The studies of Rantala et al. [3] showed that the incidence increased significantly in the high-risk newborns. The so-called high-risk newborns are infants who are prone to perinatal brain damage, cerebral palsy, mental retardation, epilepsy, and other diseases, including premature birth, low birth weight, perinatal asphyxia, fetal distress, sustained hypoxia, cranial hemorrhage, severe hypoglycemia, severe hyperbilirubinemia and other risk factors [4, 5]. The possibility of different brain developmental factors combined infantile spasms is not the same. This occurrence of spasm was often not distinguished from other normal or abnormal behavior of infants acts, abnormality of the nervous system is detected in about 2/3 of the affected infants [6]. This makes it more difficult to diagnose the infantile spasms. Although mental retardation occurs in almost all of the infants whose infantile spasms cannot be effectively controlled by drug, but this is the development qualification for the development of infantile spasms, rather than the performance of infantile spasms. However, the incidence of infantile spasms may further cause brain damage and mental damage [7]. Abnormalities (structural or functional) of the nervous system is likely to be the root causes of infantile spasms. No proper and timely treatment is given for infantile spasms and will often cause disastrous consequences [8]. How to timely discover infantile spasms and give appropriate treatment have a very significantly prognostic significance.

The latest research showed that quality assessment technology of general movements (GMs) is more sensitive and reliable as a new method for predicting early assessment of brain injury compared with the traditional neurological examination and imaging, especially having special predictive value for cerebral palsy [9, 10]. As a common complication of infants with cerebral palsy, epilepsy is known to have close relation with cerebral palsy. The researches by the domestic institutions on the correlation between general movement quality assessment as the earliest and most sensitive technology and infantile spasms (the particular form of epilepsy) has not yet been statistically analyzed. This project was carried out in the present hospital since 2009 and it was found that the infantile spasms occurred in different abnormal movement patterns in the twisting movement phase in the general movements, but the incidence was different.
In order to identify the correlation between twisting movement phase and infantile spasms, the study was performed as follows.

Materials and Methods

Experimental objects
Two hundred and one high-risk newborns experiencing neurodevelopmental follow-up during April, 2011 and April, 2012 in rehabilitation department of Xuzhou Children’s Hospital were selected; the follow-up time was more than one year. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Soochow University. Written informed consent was obtained from all participants.

Inclusion criteria: 1) GMs were recorded for at least once in twisting motion stage and 2) a clear follow-up outcome. Exclusion criteria: 1) no clear pre-production period, 2) diagnosed to be a genetic metabolic disease, 3) infantile spasms were not diagnosed in the present hospital, 4) infants lost to follow-up. A total of 178 cases were selected to be study objects with males in 112 cases and females in 66 cases, including preterm children in 98 cases (74 males and 24 females), gestational age of 28-36 weeks with mean of 32.8 ± 2.6 weeks, birth weight of 1,100 - 3,150 grams with average of 1,960 ± 566 grams.

GMs assessment
In four weeks corrected gestational age, video-recording was used to document the standardized GMs of the research subjects, with ten to 15 minutes given for each record. All video data were jointly evaluated by three evaluators. Evaluators have assessed experience of more than one year and obtained qualification certificates by participated in the training of GMs Trust. The consistency of the assessment results from three evaluators was 95%. Inconsistent assessment results were further decided by discussions and consultations of the three evaluators. Three evaluators were unaware of the basic data of the research objects.

Assessment results record
Twisting motion stage: normal GMs were recorded as N. Abnormal GMs were recorded as three subtypes including PR (monotonic), CS (spasm-synchronization) and CH (confusion).

Several evaluators took the results of the last assess GMs as the statistical materials.

Infantile spasms
Diagnosis of spasm: infantile spasms were diagnosed by neurologist in the present hospital, the diagnostic criteria included that the seizures occurred within one year of age or rarely for more than two years of age, highly imperfect EEG characteristics, often clusters of the clinical seizures, also appearing alone, and often accompanied by developmental stagnation or retrogression [11].

EEG
EEG examinations were also carried in the present hospital. The infants with abnormalities in twisting motion stage were commonly performed routine EEG, and ambulatory EEG monitoring was further performed to the newborns with abnormalities in regular EEG. Reviewed once every 45 days if the seizures were not clear and reviewed at any time if the similar seizures occurred.

Predictive validity
Predictive validity indicators included prediction sensitivity (positive proportion in the diagnostic tests for the ill persons determined by the gold standard for the diagnosis), prediction specificity (negative proportion in the diagnostic tests for the persons without illness determined by the gold standard for the diagnosis), positive predictive value (ill person proportion by the gold standard for the diagnosis in the positive diagnostic tests), negative predictive value (without ill person proportion by the gold standard for the diagnosis in the negative diagnostic tests). Statistical analysis was performed using the SPSS statistical software.

Results
A total of 178 cases study objects (male in 112 cases and female in 66 cases) were collected. The corrected gestational age was within four weeks. Eighty-three cases were recorded to be normal in GMs assessment without occurrence of infantile spasms. Ninety-five cases were abnormal, including 22 cases had the occurrence of infantile spasms (23.2%) and 73 cases without infantile spasms (76.8%). Tables 1 and 2 indicate that the positive predictive of infantile


Table 1. — Predictive validity of CS in infantile spasms.

<table>
<thead>
<tr>
<th>GMs</th>
<th>Infantile spasms</th>
<th>Without infantile spasms</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>PR+N</td>
<td>2</td>
<td>151</td>
<td>153</td>
</tr>
</tbody>
</table>

Table 2. — Different types of abnormal twisting motion and infantile spasms

<table>
<thead>
<tr>
<th>GMs</th>
<th>Infantile spasms</th>
<th>Without infantile spasms</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>2</td>
<td>68</td>
<td>70</td>
</tr>
<tr>
<td>CS</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
</tbody>
</table>

Note: The incidence of infantile spasms in the two groups was analyzed by chi-square test ($p < 0.01$). $\chi^2 = 61.6$, degrees of freedom was 1 and the difference was statistically significant.

Table 3. — Predictive validity of abnormal twisting motion in infantile spasms (cases).

<table>
<thead>
<tr>
<th>GMs</th>
<th>Infantile spasms</th>
<th>Without infantile spasms</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR+CS</td>
<td>22</td>
<td>73</td>
<td>95</td>
</tr>
<tr>
<td>N</td>
<td>0</td>
<td>83</td>
<td>83</td>
</tr>
</tbody>
</table>

Discussion

Cerebral palsy is one of the major diseases causing disability in children. Patients with cerebral palsy could be seen in all types of seizures. Epilepsy was often associated with cerebral palsy in newborns with high incidence, difficult diagnosis, and poor prognosis [12]. Epilepsy combined cerebral palsy was often difficult to control and easily transferred into intractable epilepsy and epilepticus status, which needed two or more kinds of antiepileptic drugs to treat. The epileptic seizures of these newborns will cause further damage of the existing movement, speech, and cognitive abilities, thereby rehabilitation efficacy might be damaged because of epileptic seizures. West syndrome was a severe epileptic encephalopathy of infancy with a poor developmental outcome [13]. The mortality rate was 5% to 30%; only 7.7%-16% of the normal intelligence and motor development could be restored in the survival infants, and 34.5%-68% of disease newborns had severe mental retardation and motor defects. The Primec et al. study [14] pointed out that if treatment started within a month of the spasm seizures, the seizures would be well-controlled and the development would be improved. For the newborns with low age with abnormal movement patterns, the diagnosis of epilepsy was particularly difficult and the seizures were different between infants and adults [15].

West syndrome or infantile spasms is one of the most frequent epileptic syndromes in the first year of life. The clinical symptoms of infantile spasms are very different from any other type of seizure because of both the absence of paroxysmal motor phenomena (i.e., as in a convulsion) and the lack of significant duration of loss of consciousness (i.e., as in absence epilepsy). The performance of newborn seizures might be mistaken for new parents abnormal movement patterns or muscle tension due to unclear delivery to the doctor, or newborn seizures cannot be found because of their short duration, thus epilepsy would be further aggravated. The movement intelligence, verbal, cognitive and other aspects of the infants is further damaged. The Auvin et al. study [16] also indicated that a poor outcome was related to a delay in diagnosis, which was observed regardless of the existence of cognitive involvement prior to the start of infantile spasms (relative risk: RR 12.08 [1.52 - 96.3]). These results highlighted the importance of making an early diagnosis of infantile spasms. How to perform early detection, diagnosis, and effective treatment is the top priority in the rehabilitation programs for children.

General movement quality assessment was first proposed by Austrian development neurologist Einspieler et al. in 1990 [17], which has been used as an effective tool to assess neurodevelopmental outcomes. Currently, GMs quality assessment has been widely used in foreign clinical follow-up among high-risk neonatal neurological development [18]. If the spasm synchronous systemic movement patterns continuously exist in a few weeks, they often show lack of restless movement in the development into restless movement stage, which has higher predictive value for the developmental outcomes of spastic cerebral palsy [19].
In the present study, there were 22 cases with infantile spasms (23.2%) and 73 cases without infantile spasms (76.8%) in the newborns with general motion abnormalities. The positive predictive value of general motion abnormalities in the twisting stage (PR and CS) was low (23.2%). Twenty cases with infantile spasms in the twisting motion stage had CS, accounting for 90.9%. The positive predictive value of CS movement mode for infantile spasms increased significantly (80%). The incidence of newborns with infantile spasms performing PR and CS were significantly different by statistical analysis, suggesting that the synchronization of general movement and spastic infantile spasms had a very high correlation with some predictive value, which was highly correlated between continued CS movement patterns and spastic cerebral palsy, while spastic quadriplegia and age-dependent epileptic encephalopathy had a good correlation. This study was consistent with the study results of Sugiura et al. [20].

The studies of Hrachovy and Frost [2] showed that spasms usually occurred during drowsiness or arousal from sleep and the average age was six months. In the 22 cases with infantile spasms in the present study, the youngest with onset was two months and the oldest was ten months with a mean of 5.86 months, which was consistent with the study of Hrachovy and Frost.

The present study also found that the primary factor accounting for the history of newborns with spastic synchronous movement characteristics were perinatal asphyxia, severe HIE, neonatal seizures, and low blood sugar, suggesting that severe brain injury may be the reasons for infants with spastic synchrony general movement and the poor effect of combined infantile spasms and anti-epileptic treatment [21]. This study was consistent with the study results of Gano et al. [21].

The diagnosis and treatment for infantile spasms combined with cerebral palsy were the most difficult. In diagnosis, attention should be paid to the difference with spastic synchronization general movement mode, in which the latter includes no seizures, some performances at the time of seizure such as abnormal eye movements and autonomic nerve phenomena generally did not appear in the above-mentioned types of body movement. Dynamic EEG was still indispensable in diagnosis of infantile spasms. In treatment, difficult problem was the necessity of combining the principles of antiepileptic treatment with movement disorders recovery. Epilepsy often resulted in suspension and increased dyskinesia rehabilitation. After the diagnosis of epilepsy in infants with cerebral palsy, the rehabilitation measures have to be arrested and the rehabilitation programme should be adjusted. The drug control of seizures should be the first step in a new rehabilitation program.

It should be noted that for the clear diagnosis of infantile spasms, long-term follow-up was necessary after markedly efficacy in the initial treatment. Relapse was common and the transfer into other forms of epilepsy was also seen clinically after one or two years of age [22]. In addition, newborns with spastic synchronous movement patterns may be associated with metabolic disorders at a certain degree. The anti-epileptic treatment of the consolidation of infantile spasms in these infants also required liver and kidney function assessments. As for the infants with CS motion feature but not yet infantile spasms, doctors should pay attention to reasonable and appropriate use of nerve cell nutrients in the early intervention rehabilitation and inform the possibility of merging infantile spasms and onset performance to the parents of the newborns. Regular ambulatory EEG monitoring should be performed for timely detection and treatment when infantile spasms occur in order to minimize brain damage.

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Correlation of twisting motion phase and infantile spasms in high risk infants


Address reprint requests to:
G.X. GU, M.D.
Department of Child Health,
Affiliated Childrens’ Hospital of Soochow University
No. 1166 Pingchuan Road
Suzhou 213001 (China)
e-mail: guixionggucn@163.com
Introduction

Endometriosis (EMs) is an estrogen-dependent disease characterized by the growth of endometrial stromal cells and glands outside of the uterine cavity. Between 1% and 7% of women in the general population [1] and up to 30% of women undergoing laparoscopy for chronic pelvic pain are diagnosed with endometriosis [2]. Although the cause of endometriosis remains an enigma, retrograde menstruation of shed endometrial cells and tissue fragments is thought to be central to the development of this disease. However, regurgitation of menstrual effluent occurs to some degree in all women of which only a fraction develop endometriosis. Hence, factors other than access of endometrial contents to the pelvis via retrograde menstruation are thought to contribute to the pathogenesis of this disease. However, the critical event(s) or biochemical change(s) that ultimately lead to the establishment of endometriosis remain unknown.

Most recently reported that ovarian endometriosis and adenomyosis result from the physiological mechanism of ‘tissue injury and repair’ involving local estrogen production in an estrogen-sensitive environment normally controlled by the ovary [3].

It is well known that the development and progression of endometriosis depends on the presence of estrogen. The classical human estrogen receptor, ERα, was cloned in 1986, and a second estrogen receptor, ERβ, was cloned from human testis in 1996. Both ERα and ERβ act as transcription factors and are believed to play key roles in growth regulation of the endometrium and endometriosis. Previous reports have demonstrated markedly higher levels of ERβ and lower levels of ERα in endometriotic tissues and endometriotic stromal cells. Differences in the ERα/ERβ ratio between endometriotic and eutopic endometrium could have important functional implications, since these ERs have different ligand binding characteristics [4].

Expression and significance of ERβ and TrkB in endometriosis

X. Yu¹, H. Ren², T. Liu³, M. Yong⁴, H. Zhong³

¹Department of Gynecology, Dalian Obstetrics and Gynecology Hospital Affiliated to Dalian Medical University, Dalian
²Department of General Surgery of Dalian Friendship Hospital Affiliated to Dalian Medical University, Dalian
³Department of Pathology of Dalian Friendship Hospital Affiliated to Dalian Medical University, Dalian
⁴Dalian Medical University Graduate School, Dalian (China)

Summary

Objectives: To study the potential pathogenesis of endometriosis (EMs) in an area of estrogen receptors (ERs) and tyrosine kinase receptor type B (TrkB) expressions in tissues with EMs. Study Design: The authors examined the expressions of ERα, ERβ, TrkB, brain-derived neurotrophic factor (BDNF), and SGPL1 in tissues with EMs, using real-time PCR, western blot, and immunohistochemistry. Results: ERα and SGPL1 were mainly expressed in eutopic endometrium than that in ectopic endometrium of patients with ovarian endometriosis (p < 0.05), while ERβ, BDNF, and TrkB were adverse, mainly detected in ectopic endometrium of the same patients with EMs (p < 0.01 and p < 0.05 ) by real-time PCR and western blot. ERβ, ERα, TrkB, and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase with EMs than that in eutopic endometrium of secretory phase (p < 0.05 ). TrkB, BDNF, and SGPL1 were not found in endometrium of proliferative or secretory phase in control group. Conclusions: ERβ expressed in cytoplasm may mediate pathogenesis of EMs.

Key words: Endometriosis (EMs); Estrogen receptor-α (ERα); Estrogen receptor-β (ERβ); Tyrosine kinase receptor B (TrkB); Brain-derived neurotrophic factor (BDNF); Immunohistochemistry (IHC).
show more aggressive tumorigenic and metastatic phenotype. Neurotrophins and their tyrosine kinase receptors, tropomyosin related kinase (Trk) regulate the proliferation, differentiation, and death of neuronal cells, and have been implicated in the pathogenesis and prognosis of neuroblastomas. The expression of brain derived neurotrophic factor (BDNF) and its tyrosine kinase receptor, TrkB has been correlated with clinical outcome and chemotherapy resistance in neuroblastoma. Aside from neuroblastoma, the BDNF/TrkB pathway has been shown to have an important role in a number of human malignancies such as ovarian cancer [12], Wilms’ tumor, prostate cancer, lung cancer, pancreatic carcinoma, and hepatocellular carcinoma.

In the culture of male rat-hypothalamic neurons, E2 induced an increase in the levels of TrkB. Additional experiments showed that when the E2-dependent increase of TrkB was prevented by using an antisense against mRNA for TrkB, the axogenic effect of E2 was suppressed, indicating a convergence of the signaling pathways for E2 and neurotrophins [13, 14]. Also interesting result has been reported that both mRNA and protein levels of TrkB were increased dose dependently in response to FSH treatment, implicating that FSH is one of the putative upstream mediators for TrkB expression in ovarian cancer cells [15].

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid metabolite involved in cancer development through stimulation of cell survival, proliferation, migration, and angiogenesis. Irreversible degradation of S1P is catalyzed by S1P lyase (SPL). The human SGPL1 gene that encodes SPL maps to a region often mutated in cancers [16].

For the first time, in the present study, eutopic endometrium and ectopic endometrium of patients with endometriosis were analyzed for ERs, ANOIKIS suppressor TrkB, and SGPL1 expression at mRNA and protein levels. To further probe potential mechanism for potential pathogenesis of endometriosis, revealing correlation expression of ERβ with TrkB in EM with ovarian endometriosis or adenomyosis.

Materials and Methods

Patients

Patients undergoing laparoscopy for endometriosis with ovarian endometriosis or adenomyosis were recruited for this study beginning in May 2012 in the Dalian Obstetrics and Gynecology Hospital. All tissue samples were obtained with full and informed patient consent. All patients did not receive hormonal treatments, such as GnRH agonist or sex steroids, and did not use intrauterine contraception for ≥ six months before surgery. Eighteen cases of recruited patients had regular menstrual cycles (between 26 and 32 days), had their menstrual history confirmed, and had serum 17β E2 and P levels measured just before surgery. The endometrial dating criteria as described by Noyes et al. [5] in 1950 and the menstrual history were used to assess the menstrual cycle phase. Endometrial tissue biopsies were performed just before operation. Finally, a total of 18 patients (nine patients during the proliferative phase, nine patients during the secretory phase) were selected for the present study. All patients had complaints of pain or infertility before surgery. Samples of ovarian endometriosis and adenomyosis tissues (three patients had ovarian endometriosis and adenomyosis at the same time), and the matched endometrium were divided into two portions. One part was fixed in 10% formalin, embedded in paraffin and stained with hematoxylin-eosin for pathological diagnosis. The other portion was frozen in liquid nitrogen immediately after removal and stored until use. Twelve patients with ectopic pregnancy or hysteromyoma who had tubal resection or hysteromyoma excision or uterectomy were in the control group. The study protocol followed the ethical guidelines of Dalian Friendship Hospital. Informed consent was obtained from all subjects.

RNA isolation and real-time PCR

Total RNA was prepared by using TRizol. Primers for cDNA amplification were as follows: ERα (F- TCTGGCAAGGA-GACTCGCTA, R-CTTTTCGTATCCCACTTTTACAT 241bp), ERβ (F-GATTAGGGGAATGCGTGAAAG, R-GGCAATCACC-CAAACCAAAG 234bp), TrkB (F- TTACCGGAAAAACACT-GAGCA, R-GTCTGGACTGATTAGCCTTCT, 146bp), BDNF (F- TGGAGGCTATGTGAGTTGG, R- GGCGAATGTGCGT-GCTTGAGT 257bp), SGPL1 (F-TCGGTGAGAAGCGCTATGT, R- CGGGCGTGTAGTAATGTGAT 247bp), and GAPDH (F- GGCAATTGGGAACGTGCTG, R- GAGTGGGTGTCGCT-GTTGAAG 257bp). Real-time PCR was used using SYBR Premix Ex Taq. All reactions were carried out according to the manufacturer’s protocols. The primer sequences were as above. The annealing temperature for these primer sets was 60°C. The specificity of each PCR reaction was confirmed by melting curve analysis. The level of target gene expression in each sample was normalized to the respective GAPDH expression level [17].

Immunohistochemistry

Tissue samples were fixed, cut, mounted, deparaffinized and rehydrated. For endogenous peroxidase quenching, the slides were incubated in 0.3% hydrogen peroxide (H₂O₂) for ten minutes. After blocked with goat serum, the sections were incubated with the primary antibody solution at 4°C overnight (1:100 dilution for ERα, 1.70 dilution for TrkB, 1.100 dilution for BDNF, and 1.200 dilution for SGPL1, and 1:200 dilution for ERβ). The slides were rinsed twice with PBS and incubated with peroxidase-conjugated secondary antibodies for one hour at room temperature. After rinsing, color was developed by incubating slides with three, 3-diaminobenzidine for five minutes followed by counterstaining with hematoxylin for ten seconds. The slides were dehydrated with successive washes of dH₂O, 95% and 100% ethanol, and xylene before mounting with cover slips. The breast cancer and ovarian cancer specimens were used as positive control, while the primary antibodies was replaced with PBS as negative control. Immunohistochemical staining results were evaluated by blind method. Nuclear immunoreactivity with ERα, nuclear, and cytoplasmic immunoreactivity with ERβ, cytomembrane, and cytoplasmic immunoreactivity with TrkB, and cytoplasmic immunoreactivity with BDNF and SGPL1 were considered as positive.

Western blot analysis

After rinsed twice with ice cold PBS, the cells were scraped and lysed in ice-cold HNTG buffer [50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1.5 mmol/L MgCl₂, one mmol/L EDTA, ten mmol/L sodium PPI, 100 μmol/L sodium orthovanadate, 100 mmol/L NaF, ten μg/ml aprotinin, ten μg/ml leupeptin, and one mmol/L phenethylsulfonyl fluoride] on ice for 30 minutes. Total proteins were measured Bio-Rad protein assay reagent according to the manufacturer’s protocol. Twenty μg protein was separated on 12% of SDS-PAGE gels and transferred to nitrocellulose membrane. After blocked with 10%
bovine serum albumin in 1×Tris-buffered saline, the membrane was incubated with various primary antibody against ERα, TrkB, BDNF, SGPL1, ERβ, and GAPDH at 4°C overnight. The membrane was washed with PBS three times, and then incubated with peroxidase-linked secondary antibody (1:10000) for one hour at room temperature. The signals were developed with the ECL kit, scanned, and analyzed with TotalLab software. The relative expression of target proteins was presented as the ratio to β-actin or GAPDH.

Statistics

Results are presented as mean ± SD. Data were analyzed with paired Student’s t test for comparison between groups. A p < 0.05 was considered statistically significant.
Figure 3. — Expressions of TrkB, BDNF, and SGPL1 proteins of patients with ovarian endometriosis or adenomyosis were determined by IHC. (A, B) Cytoplasmic staining of TrkB protein was detected in eutopic endometrium of proliferative phase. (C) Cytoplasmic staining of TrkB protein was detected in eutopic endometrium of proliferative phase, at lower power. (D) TrkB protein was detected in eutopic endometrium of secretory phase. (E) TrkB protein was not detected in eutopic endometrium of secretory phase. (F, G) Cytoplasmic staining of TrkB protein was detected in ectopic endometrium of ovarian endometriosis. (H) Cytoplasmic staining of TrkB protein was detected in functionalis glands of adenomyosis. (I) Cytoplasmic staining of BDNF protein was detected in ectopic endometrium of ovarian endometriosis. (J) Cytoplasmic staining of BDNF protein was detected in functionalis glands of adenomyosis. (K) Cytoplasmic staining of SGPL1 protein was detected in ectopic endometrium of ovarian endometriosis. (L) Cytoplasmic staining of SGPL1 protein was detected in functionalis glands of adenomyosis (maximal magnification ×400).

Figure 4. — Expressions of ERα and SGPL1 proteins in ectopic and eutopic endometrium of patients with ovarian endometriosis were determined by western blot. Western blot analysis shows the bands demonstrating ERα protein (66 kDa) and SGPL1 protein (63 kDa) in ectopic endometrium, eutopic endometrium, and proliferative or secretory phase of eutopic endometrium of patients with ovarian endometriosis differently \( p < 0.05 \). (Ov and ECE represent ectopic endometrium; En and EuE represent eutopic endometrium. Pro-En represent eutopic endometrium of proliferative phase, Sec-En represent eutopic endometrium of secretory phase).

Figure 5. — Expressions of ERβ and TrkB proteins in ectopic and eutopic endometrium of patients with ovarian endometriosis were determined by western blot. Western blot analysis shows the bands demonstrating TrkB protein (145 kDa) and ERβ protein (59 kDa) in ectopic endometrium, eutopic endometrium, and proliferative or secretory phase of eutopic endometrium of patients with ovarian endometriosis differently \( p < 0.05 \). (Ov and ECE represent ectopic endometrium; En and EuE represent eutopic endometrium. Pro-En represent eutopic endometrium of proliferative phase, Sec-En represent eutopic endometrium of secretory phase).
Expression and significance of ERβ and TrkB in endometriosis

Results

Expression of ERα, ERβ, TrkB, BDNF, and SGPL1 in eutopic and ectopic endometrium of patients with ovarian endometriosis

The authors observed ERα and SGPL1 mRNAs were mainly expressed in eutopic endometrium than that in ectopic endometrium of the same patient with ovarian endometriosis (p < 0.05). ERβ, BDNF, and TrkB mRNAs were notably detected in ectopic endometrium than in corresponding eutopic endometrium (p < 0.01 and p < 0.05 ) by real-time PCR. The authors also found high ratios of ERβ mRNA and ERα mRNA levels in endometriotic tissues of ovarian endometriosis (Figure 1). At protein level, certified that ERα and SGPL1 proteins were dominantly expressed in eutopic endometrium, while ERβ and TrkB proteins were detected mainly in eutopic endometrium by western blot (p < 0.05 ) (Figure 4). Importantly ERβ, ERα, TrkB, and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase with ovarian endometriosis than that of secretory phase by western blot (p < 0.05 ) (Figures 4 and 5). TrkB, BDNF, and SGPL1 were not found in proliferative or secretory phase endometrium of control group (data not shown). ERα and ERβ were expressed in proliferative or secretory phase endometrium of control group normally.

Expression of ERα, ERβ, TrkB, BDNF and SGPL1 proteins in eutopic and ectopic endometrium of patients with EMs by IHC

IHC staining showed that nuclear staining of ERα and cytoplasmic staining of ERβ in eutopic and ectopic endometrium, while TrkB, BDNF, and SGPL1 proteins were detected cytoplasmic staining in eutopic and ectopic endometrium of patients with EMs. Especially the authors certified that ERβ, ERα, TrkB and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase with ovarian endometriosis or adenomyosis by IHC (Figures 2 and 3). In addition, the present study demonstrated positive staining of ERα, ERβ, TrkB, BDNF, and SGPL1 proteins in functionalis glands of adenomyosis.

Discussion

Endometriosis is a benign lesion with malignant ability, and the incidence reaches up to 80% in infertile women. EMs may be associated with localized high concentration of estrogen and abnormal enhancement of ANOIKIS suppression and migration, but the mechanism is not yet elucidated. Although the exact mechanism for the development of endometriosis remains unclear, there is a large body of research data and circumstantial evidence that suggests a crucial role of estrogen in the establishment and maintenance of this disease.

Despite its sensitivity to estrogen, endometriosis appears to contain a unique but severely altered complement of steroid hormone receptors compared with that of its normal tissue counterpart, eutopic endometrium. Moreover, a number of investigators have reported markedly elevated levels of estrogen receptor ERβ and lower levels of ERα in human endometriotic tissues when compared with eutopic endometrial tissues [19]. The present results also revealed that higher ratios of ERβ and ERα mRNA levels in ectopic endometrium than that in eutopic endometrium from ovarian endometriosis patients by real time PCR.

Unlike ERα, ERβ plays a minor role in mediating estrogen action in the uterus, on the hypothalamus/pituitary, the skeleton, and other classic estrogen target tissues. However, a clear role for ERβ has been established in the ovary, cardiovascular system, and brain as well as in several animal models of inflammation including endometriosis [20]. The high ratios of ERβ and ERα levels in endometriotic stromal cells in turn lead to increased ERβ binding to the PR promoter and mediates the downregulation of expression of PR [21].

In light of endometriosis’s malignant behaviors, the authors detected expression of SGPL1 and TrkB which may relate to oncogenesis of human cancer in endometriosis tissues.

By affecting S1P metabolism and the expression of Bcl-2 members, the loss of SPL enhances cell resistance to anticancer regimens and results in an increased ability of cells to acquire a transformed phenotype and become malignant [22]. For the first time the authors found that SGPL1 was expressed in endometrium of patients with EMs, as ERα, the higher level was in eutopic endometrium than that in ectopic endometrium of ovarian endometriosis. There is fewer research regarding expression of TrkB in EMs reported. Expression of TrkB was found upregulated of TrkB in epithelial cells from deep infiltrating endometriosis (DIE), which might be involved in molecular mechanisms of perineural and intraneural invasion [11]. A study detected TrkB mRNA in epithelial cells from ovarian endometriosis patients [23]. The expression levels of TrkB in epithelial cells from DIE were significantly decreased in patients with preoperative GnRH agonist or progestin. However, the functional roles of TrkB in DIE remain to be clarified [24]. In this study, for the first time the authors found that expression of TrkB mRNA and protein and its ligand BDNF was detected in eutopic and ectopic endometrium tissues from ovarian endometriosis patients, and the higher levels were revealed in ectopic endometrium than that in eutopic endometrium notably by real time PCR and western blot.

To certificate the results above, the authors examined expression of ERα, ERβ, TrkB, BDNF, and SGPL1 proteins in eutopic and ectopic endometrium from patients with ovarian endometriosis or adenomyosis by IHC. IHC staining showed that nuclear staining of ERα and cytoplasmic staining of ERβ in epithelial cells, and cytoplasmic staining of TrkB, BDNF, and SGPL1 protein in epithelial cells of eutopic and ectopic endometrium with ovarian en-
dometriosi or adenomyosis. Whether there are differences in the expressions of some proteins through the menstrual cycle in uteri with and without EMs or adenomyosis is a interesting topic. For the first time the authors revealed that ERβ, ERα, TrkB, and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase other than secretory phase with ovarian endometriosis or adenomyosis, and TrkB, BDNF, and SGPL1 were not found in proliferative or secretory phase endometrium of control group (data not shown). This result confirmed that distinct protein expression in eutopic endometrium of women with EMs, and the molecular changes in eutopic endometrium of proliferative phase would more crucial for endometrial cells destined to become endometriotic implants of women with endometriosis.

The present study also demonstrated that nuclear or cytoplasmic staining of ERα, ERβ, TrkB, BDNF, and SGPL1 proteins were detected in functionalis glands of adenomyosis [25]. It is reported that the ERα expression in the adenomyotic endometrium was different from that of the normal endometrium and the foci in the mid secretory phase of the cycle, but expression of ERα in the inner and outer myometrium was not statistically significantly different. The ERβ expression was statistically significantly elevated in the adenomyotic functionalis gland during the proliferative phase and throughout the myometrium across the entire menstrual cycle.

In humans, however, the roles of ERα and ERβ on proliferation and apoptosis in endometrium and endometriosis are not well known; ERα regulation of cell cycle in eutopic endometrium is probable. High levels of ERβ suppress ERα expression and response to estradiol in endometrial and endometriotic stromal cells via binding to classic and nonclassic DNA motifs in alternatively used ERα promoters. ERβ also regulates cell cycle progression and might contribute to proliferation of endometriotic stromal cells [18]. The present results showed the similar expression pattern of ERβ and ANOIKIS suppressor TrkB in EMs. Unlike ERα, ERβ showed nuclear staining by IHC. The significance of this cytoplasmic staining is not yet clear, however, the earlier findings on ERα may provide an analogical interpretation for localization of ERβ in cytoplasm or/and vesicle: a subpopulation of ERα was localized in caveolae [26]; antiapoptotic signal was mediated by ERα when ERα was localized on the membrane, while this signal was lost when ERα was in the nucleus [27]. Recently, this signaling above mediated by membrane associated ERα and ERβ has been termed nongenomic signaling and more recently non-nuclear signaling [28, 29]. ERα and ERβ are found both in association with the plasma membrane (not transmembrane), in the cytoplasm and in the nucleus. Estrogen bound membrane associated ERα and ERβ can each activate a signaling cascade that includes PI3K and Akt, as well as ERK 1/2, JNK and p38. This signaling cascade protects the cell from injury, except for JNK, which increases apoptosis. Interestingly in this study the author used breast cancer and ovarian cancer specimens as positive control for ERα and ERβ protein detection (IHC), ERα was not observed in these specimens; ERβ was demonstrated with nuclear staining in ovarian carcinoma, and cytoplasmic and membranous staining in breast carcinoma simultaneously.

Full-length TrkB is a 145kDa transmembrane protein, preferentially activated by brain-derived neurotrophic factor (BDNF). Following ligand binding, TrkB forms homodimers resulting in auto-phosphorylation on tyrosine residues, which is required for its catalytic and signaling activities. TrkB also could be auto-phosphorylated independent of BDNF stimulation in ovarian cancer cells, with the PI3K/AKT activation mediated by TrkB overexpression, the ovarian cancer cells showed high ability of chemotherapy-resistance and metastasis [12].

Although few studies assessed the relationship between E2/ERs and TrkB/BDNF expression in cancers or EMs, but a clue was revealed by Cheung et al. [30]: FSH is one of the putative upstream mediators for TrkB expression in ovarian cancer cells. If it could be presumed that the nonnuclear signaling of E2/ERβ activation in EMs may upregulate TrkB expression, with the PI3K/AKT activation mediated by TrkB overexpression, the endometriotic cells showed high ability of proliferation, ANOIKIS suppression, and invasion. Moreover, the potential mechanism of FSH/E2 inducing TrkB expression and activation mediated by the nonnuclear signaling of E2/ERs needs to be further investigated.

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Address reprint requests to:

X. YU, M.D.
Department of Gynecology, Dalian Obstetrics and Gynecology Hospital
Affiliated to Dalian Medical University
No. 33 Dunhuang Road
Dalian, 116033 (China)
e-mail: yuxiaohui369@163.com
Histopathology of ipsilateral and contralateral ovaries and plasma interleukin 6 levels after unilateral ovarian torsion

L. Karakoc-Sokmensuer1,2, S. Hacivelioglu3, A. Demir4, M. Köse5, F.F. Kaymaz1, D.U. Cakir6, G. Bozdag2
1 Department of Histology and Embryology, School of Medicine, Hacettepe University, Ankara
2 Department of Obstetrics and Gynecology, School of Medicine, Hacettepe University, Ankara
3 Department of Obstetrics and Gynecology, School of Medicine, Onsekiz Mart University, Canakkale
4 Elazığ Training and Research Hospital, Department of Obstetrics and Gynecology, Elazığ
5 Afyon State Hospital, Department of Obstetrics and Gynecology, Afyon
6 Department of Clinical Biochemistry, School of Medicine, Onsekiz Mart University, Canakkale (Turkey)

Summary
Objective: The aim of the present study was to evaluate the time-dependent histopathologic changes in both ovaries and to determine the time-dependent levels of plasma interleukin 6 (IL-6) after unilateral ovarian torsion. Materials and Methods: An experimental animal study included 48 female Sprague-Dawley rats which were distributed to six groups: control group (Group 1), sham-operated control group (Group 2), and four unilateral ovarian torsion groups with torsion duration of three, six, 12, and 24 hours (Group 3, 4, 5, and 6, respectively). Histopathologic criteria (follicular degeneration, vascular congestion, hemorrhage, inflammatory cell infiltration, and total tissue damage score) were evaluated in both ovaries, and plasma IL-6 levels were measured. Results: At 24 hours after torsion began, mean total tissue damage score was similar between ovaries that had torsion and contralateral ovaries. Mean plasma IL-6 level did not change during the 24 hours after torsion began (p = 0.584). Conclusions: In addition to ovaries that had torsion, histopathologic abnormalities also occurred in contralateral ovaries. These results suggest that contralateral ovaries are not quiescent after unilateral ovarian torsion. Plasma IL-6 levels did not change significantly during the 24 hours after ovarian torsion began, resulting in a limitation of its diagnostic use in the early course of the disease.

Key words: Acute abdominal pain; Surgical emergency; Biomarkers; Cytokines.

Introduction
Ovarian torsion is a rare but important surgical emergency that comprises 2.7% of gynecologic emergencies [1]. Torsion of the ovarian vascular pedicle causes ischemia and infarction because of compromised blood supply to the ovary and obstruction of the venous and lymphatic drainage. Therefore, early accurate diagnosis with prompts treatment is crucially important to salvage ovarian tissue and preserve future fertility. However, the diagnosis of ovarian torsion mostly relies on clinical suspicion and non-specific clinical and laboratory findings, and incorrect or late diagnosis may occur. An accurate preoperative diagnosis is made in only 38% of patients, and definitive diagnosis usually is established only by intraoperative findings [2].

Histologic and ultrastructural changes may occur in the contralateral ovary after unilateral ovarian ischemia [3]. However, the time-dependent pattern of the histologic changes that develop within both ovaries after unilateral ovarian torsion has not been clearly investigated. There is no specific laboratory biomarker for the preoperative diagnosis of ovarian torsion. A specific laboratory biomarker may improve diagnosis and minimize delayed or incorrect diagnosis. Interleukin 6 (IL-6) is a proinflammatory cytokine and an acute phase protein. Serum IL-6 levels increase during ischemic events such as myocardial and intestinal ischemia [4, 5]. In addition, IL-6 levels are increased in ovarian torsion and may be helpful in making a preoperative diagnosis [6-9]. However, the pattern and extent to which IL-6 levels increase in the early course of ovarian torsion are unknown. Such information may improve the specificity and usefulness of this serum biomarker in early preoperative diagnosis.

The purpose of this experimental study was to evaluate time-dependent histopathologic changes in both ovaries and determine the time-dependent levels of IL-6 after unilateral ovarian torsion.

Materials and Methods
This study was performed with 48 female Sprague-Dawley rats (weight: 200 to 220 grams). Before the experiment, the rats were maintained in standard laboratory conditions. Animals were randomly distributed to six groups (eight rats each): control group...
out laparotomy (Group 1), sham-operated control group (Group 2), and four unilateral ovarian torsion groups with torsion duration of three, six, 12, and 24 hours (Group 3, 4, 5, and 6, respectively). The researchers who performed histologic and biochemical studies were blinded to the randomized group identification until the end of the study. The study was approved by the ethical committee of the Hacettepe University, Medical Faculty and the ethical guidelines for care and use of the animals in experimental studies were followed.

Laparotomy was performed in all except in the Group 1 rats (only blood was obtained for IL-6 measurements) and unilateral ovarian torsion was created in all except in the Group 1 and 2 rats. Each rat was anesthetized with ketamine hydrochloride (50 mg/kg, intraperitoneal) and xylazine hydrochloride (ten mg/kg, intraperitoneal). Rats were placed in a dorsal recumbent position and covered with sterile drapes before surgery. The skin area for the incision was shaved and disinfected. A longitudinal lower abdominal incision (length: two cm) was made and both uterine horns and ovaries were identified. In the sham-operated control group, the abdomen was opened and both ovaries were surgically removed without torsion. In the remaining four study groups, unilateral left ovarian torsion was created by using vascular clips just above and below the left ovary as previously described [10]. The abdomen was surgically closed in two layers with silk 3-0 sutures.

At three, six, 12, or 24 hours after ovarian torsion in the four study groups, repeated laparotomy was performed through the previous incision and both ovaries (left ovary with torsion; contralateral right ovary without torsion) were removed for light and electron microscopic evaluation. Immediately after the ovaries were excised, blood (two to three ml) was obtained by aspiration from the heart for determination of plasma IL-6 levels, and the rat was sacrificed.

Ovaries were rapidly fixed in 10% phosphate-buffered formalin, dehydrated in graded alcohols, and processed for light microscopy. All specimens were embedded in paraffin blocks. Sections (five µm thickness) were cut and stained with hematoxylin-cosin and Masson trichrome stains. Sections were examined with a light microscope and photographed.

A single histologist assessed the histologic changes in a blinded manner. The four criteria for ovarian histopathologic injury were follicular degeneration, vascular congestion, hemorrhage, and inflammatory cell infiltration. Each specimen was scored semiquantitatively for each criterion with a scale ranging from 0 to 3 (0: none; 1: mild; 2: moderate, and 3: severe) as previously described [11]. The total tissue damage score was defined as the sum of the scores of the four histologic parameters (total range, 0 [no damage] to 12 [most severe damage]).

For electron microscopy, tissue samples were immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for two hours at room temperature. After washing three times for ten minutes in 0.1 M phosphate buffer (pH 7.4), samples were postfixed with 1% osmium tetroxide. After dehydrating in an ethanol gradient at room temperature, the tissue samples were infiltrated and embedded in epoxy resin. For light microscopy, semithin (one µm thickness) sections were stained with toluidine blue-azure II stain before examination. An ultramicrotome was used to cut two blocks per ovary. The ultrathin (70 nm thickness) sections were double-stained with uranyl acetate and lead citrate before viewing with an electron microscope operating at 80 kV.

All blood samples were studied simultaneously in the same assay, and the laboratory staff members were blinded to the identity of the samples (control, sham-operated or study groups). For plasma IL-6 measurements blood samples collected in tubes with ethylenediaminetetraacetic acid were centrifuged for 15 minutes at 2,000 g at room temperature.

The plasma was immediately stored in pyrogen-free tubes at -80°C until use. The IL-6 plasma levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to instructions from the manufacturer. The wells of microtiter strips were coated with antibodies specific to rat IL-6. The samples and known rat standards were added to the wells, incubated, and washed. The intensity of absorbance was determined at 450 nm with an ELISA reader. The IL-6 levels were expressed in pg/ml.

Data analysis was performed with statistical software (SPSS version 20). Data were reported as mean ± SD. The data were assessed with visual (histogram and probability plot) and analytical methods (Shapiro-Wilk test) to evaluate normal distribution. The non-parametric Kruskal-Wallis test was used to compare scores for follicular degeneration, vascular congestion, hemorrhage, and inflammatory cell infiltration at the different times because these variables were not normally distributed. After performing the Kruskal-Wallis test, the Mann-Whitney test was performed to test the significance of the pairwise differences, using a Bonferroni adjustment for multiple comparisons. Plasma IL-6 levels were normally distributed between the study groups, and one-way analysis of variance was used to compare IL-6 levels between the groups. The Mann-Whitney test was applied to compare the total tissue damage score between the ipsilateral and contralateral ovaries at different times. Statistical significance was defined by $p \leq 0.05$. 

Figure 1. — Light micrographs of rat ovaries. A) In sham-operated control rats, normal follicular structure with different stages of developing follicles and no pathologic changes were noted (Masson trichrome, original magnification ×20). B) At 12 hours after ovarian torsion, the ovary with torsion had severe follicular degeneration, severe vascular congestion, and severe hemorrhage (Masson trichrome, original magnification ×5). C) At 24 hours after ovarian torsion, the contralateral ovary had severe follicular degeneration, moderate vascular congestion, severe hemorrhage, and moderate inflammatory cell infiltration (Masson trichrome, original magnification ×10).
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Results

On gross inspection, most ovaries that had torsion had a cherry-red to black color and appeared hemorrhagic and edematous. Histologic examination of the sham-operated ovaries showed normal ovarian structure (Figure 1A). In all ovaries from rats that had ovarian torsion, there were various degrees of follicular degeneration, vascular congestion, hemorrhage, and inflammatory cell infiltration both in torsioned and contralateral side (Figures 1B and C). In all ovaries that had torsion, there were free-floating follicular cells within the antral space. In most rats that had ovarian torsion, the torsioned ovaries showed a decreased number of follicular cell layers and enlarged intercellular spaces between follicular cells.

In the ovaries that had torsion, the scores of all four histopathologic parameters increased significantly with time after torsion. The contralateral ovaries had a significant increase in three histologic parameters (follicular degeneration, vascular congestion, and infiltration), but there was no significant change in hemorrhage. The torsioned ovaries had mean total tissue damage scores higher from contralateral ovaries in three, six, and 12 hours; however, the mean total tissue damage scores were similar at 24 hours between torsioned and contralateral ovaries (Table 1).

Electron microscopic examination showed normal ovarian ultrastructure in the sham-operated group (Figure 2A). At 12 hours after torsion began, the ovaries that had torsion had separated granulosa cells with enlarged granulated en-
doplasmic reticulum, distorted perinuclear cisternae (accumulation of electron lucent material), and lipid droplets (Figures 2B and C).

Mean plasma IL-6 levels did not change significantly from 0 to 24 hours after torsion began (Figure 3).

Discussion

The present results showed that histopathologic changes of similar severity occurred by 24 hours after torsion, both in torsioned and in contralateral ovaries. There were distinctive ultrastructural changes noted in the torsioned ovaries after 12 hours. In addition, plasma IL-6 levels did not change significantly during 24 hours after ovarian torsion.

Ischemia and histopathologic changes were expected to be more severe in torsioned ovaries than contralateral ovaries. However, the mean total tissue damage score was higher in torsioned ovaries than contralateral ovaries only from three to 12 hours, and after 24 hours the score was similar between torsioned and contralateral ovaries (Table 1). A previous study that evaluated ischemia-modified albumin as a marker of ovarian torsion also showed significantly higher total tissue damage scores after three hours from torsion [12]. Another study that assessed the protective effect of the drug iloprost in an ovarian torsion model showed that the ipsilateral ovaries that had torsion had significantly higher total tissue damage scores than the rats without torsion after four hours [13]. However, the study that evaluated iloprost showed no significant change in the mean total tissue damage score in the contralateral ovaries between the animals that had torsion and control animals after four hours [13]. In addition, contrary to the present findings, another study of unilateral ovarian torsion showed that the histology of the contralateral ovaries was unaffected, with normal ovarian cortices, from four to 36 hours after torsion; histologic sections of the ovaries that had torsion had negligible changes, with an intact ovarian structure similar to controls in the four to 24 hour groups [14]. In contrast, the present study showed that all histopathologic parameters except hemorrhage in contralateral ovaries changed significantly within 24 hours after torsion for both torsioned and contralateral ovaries (Table 1). The discrepancy between studies may have occurred because of different examiners and histologic parameters in different studies.

There are some studies that concluded that histologic, ultrastructural, and functional changes may develop in the contralateral, uninvolved ovary after unilateral ovarian torsion.
 torsion [14, 15]. The present study showed that histopathologic changes are similar between the torsioned and contralateral ovaries after 24 hours. The mechanisms for these changes in the contralateral ovary are unknown. After unilateral ovarian torsion, histologic and ultrastructural changes and decreased hormone production in the contralateral ovary may occur because of neuroendocrine and neurorregulatory mechanisms [14, 15]. Tissue ischemia in the torsioned ovary may stimulate the sympathetic nervous system, and this may decrease regional blood flow and cause hypoxia in the contralateral ovary [15, 16]. There may be an indirect or direct connection between the sympathetic nervous systems of the ovaries. After unilateral testicular torsion, a similar reduction in blood flow to the contralateral testis occurs, with a mechanism that may be similar to the mechanism in ovarian torsion [17]. The results of the present study suggest that the hypoxia caused by sympathetic activation may cause histopathologic changes in the contralateral ovary. However, further experimental and clinical studies are necessary to clarify the mechanisms that cause histopathologic and functional changes in the contralateral ovary.

The proinflammatory cytokine IL-6 may be another serum marker of ovarian torsion. However, the present study showed no significant change in plasma IL-6 levels during the first 24 hours after torsion (Figure 3). Previous clinical studies had suggested that plasma IL-6 levels may have diagnostic value in ovarian torsion and may facilitate prompt diagnosis and treatment [7-9]. When clinical signs of ovarian torsion are inconclusive, serum IL-6 level might help to distinguish the patients that should undergo diagnostic laparoscopy [9]. A meta-analysis of the previous studies showed increased serum IL-6 levels in patients who had ovarian torsion, and serum IL-6 level may have good sensitivity (86%) and positive likelihood ratio for the diagnosis of ovarian torsion in patients who have abdominal pain and ultrasonographic evidence of an ovarian cyst [6, 8, 9]. The present study was limited to 24 hours after ovarian torsion; it is possible that IL-6 levels may increase after 24 hours torsion.

The absence of a change in plasma IL-6 levels observed in this study (Figure 3) was different than previous findings in rats with ovarian torsion. In a previous experimental study, the study design was similar to the current study and serum IL-6 levels were measured at 0 and 3 hours after ovarian torsion. A significant increase in serum IL-6 level was observed at three hours after torsion [18]. Another study also reported significantly elevated serum IL-6 levels at three hours after ovarian torsion [19]. It is unknown why the present and previous studies had different results because the study designs and laboratory methods were similar for the first three hours after torsion. Further studies may clarify the time-dependent changes and usefulness of IL-6 plasma levels in the early diagnosis of ovarian torsion.

Limitations of this study include the animal model that may not necessarily simulate ovarian torsion in clinical practice. Serial blood measurements from the same animal were not performed after ovarian torsion and may be useful in evaluating changes in IL-6 levels. In addition, the study was limited to 24 hours after ovarian torsion, and measurements after 24 hours were not performed. Nevertheless, the study extends the available information about ovarian torsion because it included time-dependent data about plasma IL-6 levels, which may be useful in the early and correct diagnosis of the disease.

In conclusion, the present study showed that histopathologic changes were similar in torsioned and contralateral ovaries at 24 hours. Therefore, contralateral ovaries are not quiescent after unilateral ovarian torsion. In addition, plasma IL-6 levels did not change significantly during the 24 hours after ovarian torsion, and it is unknown whether IL-6 levels may increase after 24 hours. More studies are needed to reveal the time-dependent changes in IL-6 levels after 24 hours.

References


Address reprint requests to:
L. KARAKOC-SOKMENSUER, M.D.
Hacettepe University, Medical School
Geuher Nesibe Street no. 1
06100 Sihhiye, Ankara (Turkey)
e-mail: lalekarakoc@hacettepe.edu.tr
Introduction

The rate of multiple pregnancies in developed countries continues to rise as a consequence of assisted conception and increasing maternal age [1]. In the United States, between 1980 and 2007, the twin rate climbed 101% [2]. In Italy, between 1990 and 2005, the number of twin births has increased by 25% and twin gestation today represents 1.5% to 3% of all pregnancies. About 80% of twin pregnancies are dichorionic (DC) and 20% are monochorionic (MC) [3]. Chorionicity is the most important determinant of mortality and morbidity in twins. Most studies confirmed higher risks for MC compared with DC pregnancies in relation to perinatal outcomes [4, 5]. Indeed, the main risks for MC twins are the twin-twin transfusion syndrome (TTTS) (15%) [6] and prematurity (50%) [7]. However, the recent rise in twin pregnancies results mainly from a greater proportion of DC pregnancies, that is largely explained by the increasing use of assisted reproduction technology (ART). In the USA and Europe between 20% and 30% of deliveries following ART are twins, compared with approximately 1% following spontaneous conception (SC) [8]. In Caucasian populations, about 22% of SC twins are MC, whereas this rate falls to about 2% in ART [9]. Literature data concerning the influence of ART on the outcome of twin pregnancies are controversial.

The purpose of this study was to evaluate the maternal and neonatal outcomes in multiple pregnancies, according to two different parameters, namely chorionicity (MC versus DC) and mode of conception (SC versus ART).

Materials and Methods

The authors performed a retrospective analysis of the multiple pregnancies that delivered at the Department of Gynecology, Obstetrics and Urology of the University of Rome Sapienza, from January 2008 to April 2013. Exclusion criteria were gestational age (GA) less than 24 weeks at delivery and early or late miscarriages.

The authors acquired the details of the pregnancies and deliveries from the medical records. In particular, they collected demographic data, relevant past history, parity, mode of conception (SC versus ART), chorionicity (MC versus DC), GA at admission and at delivery, time of maternal hospital stay ante- and post-partum, pregnancy outcomes, and maternal-fetal complications, therapy during gestation, short-term neonatal outcomes.

The chorionicity was determined at 10-14 weeks on the basis of the presence or absence of the lambda sign or a projection of choriodecidual tissue into the inter twin membrane. Gestational age was calculated from the last menstrual period and confirmed, or corrected, by means of ultrasounds examination through the measurement of the crown-rump length.

The authors recorded the following maternal-fetal complications: preterm delivery, threatened preterm labour, preterm premature rupture of membranes (pPROM), intrauterine growth restriction (IUGR), TTTS according to Quintero’s criteria [10], and selective IUGR, pregnancy induced hypertension (PIH), preeclampsia (PE), hemolysis, elevated liver enzyme levels, and low platelet count (HELLP) syndrome, gestational diabetes mel...
litus (GDM), cholestasis, oligohydramnios, polyhydramnios, abnormal placentation, placental abruption, and intrauterine death (IUD).

With regards to short-term neonatal outcome, the authors collected the following parameters: birth weight and related percentile according to classification proposed by Parazzini et al. [11], Apgar score at one and five minutes, and the discordance of twin weight. Discordance was calculated by using the following formula: larger twin – smaller birth weight x 100/larger twin birth weight.

Patients were grouped according to chorionicity (MC vs DC) and the mode of conception (SC vs ART), to evaluate the impact of these variables. The data were analyzed with SPSS 16.5 Windows program. Comparisons between the two groups were performed with the Student’s t-test and chi-square test. Significance was assumed at \( p < 0.05 \).

### Results

The authors collected 196 twin pregnancies, further subgrouped into 55 MC and 141 DC. The MC subgroup included 49 SC pregnancies (89.1%) and six conceived by ART (10.9%). In particular, three pregnancies were obtained by intracytoplasmic sperm injection (ICSI), one pregnancy by fertilization in vitro and embryo transfer (IVF-ET), and two pregnancies after intrauterine insemination (IUI). The DC subgroup included 82 SC pregnancies (58.2%) and 59 conceived by ART (41.8%). In particular, 35 pregnancies were obtained by ICSI, 21 pregnancies by IVF-ET, and three pregnancies by IUI. The authors considered only the 36 DC pregnancies obtained by IVF, namely IVF-ET and ICSI, after ovulation induction in order to compare SC versus ART twin pregnancies.

#### MC versus DC pregnancies

The authors compared 55 MC versus 141 DC pregnancies. Maternal age, parity, duration of ante and post-partum hospital stay, and type of delivery, were not significantly different between subgroups.

With regards to the mode of conception, the rate of conception by ART was significantly higher in the DC subgroup (41.8% vs 10.9%; \( p < 0.05 \)). GA at admission was approximately two weeks earlier in MC pregnancies (33 ± 3 vs 35 ± 2.7 weeks, \( p < 0.05 \)).

The incidence of abnormal placentation, placental abruption, IUD, PIH, PE, HELLP syndrome, cholestasis, and GDM was not significantly different between the two subgroups. The incidence of threatened preterm delivery was 58.2% vs 32.6% \( p = 0.001 \) [OR= 2.87, 95% CI 1.51 - 5.46], and pPROM was 31% vs 13.5% \( p = 0.007 \) [OR= 2.87, 95% CI 1.36 - 6.07]. Moreover, 7.2% of MC pregnancies were complicated by TTTS, while this complication never occurred in the DC subgroup \( p < 0.05 \). No case of TTTS was treated with laser therapy prior to delivery.

GA at delivery was on average one week earlier in MC group than DC group (34±3 vs 35.5 ± 2.3 weeks). In particular, 85.5% of MC delivered before 37 weeks, as compared to 66.7% in the DC subgroup \( p < 0.05 \) [OR= 2.94, 95% CI 1.28 - 6.72].

In the MC subgroup, IUGR occurred in 28% of cases, as compared to 12% in the DC subgroup \( p < 0.05 \), though the difference of incidence of selective IUGR did not reach statistically significance. In MC pregnancies, lower neonatal birth weight were found (1,990 ± 526 vs 2,246 ± 473 grams).
The impact of chorionicity and mode of conception on maternal-neonatal outcome in twin pregnancies

p < 0.05, as well as lower birth weight percentile (38.7 ± 26 vs 46.4 ± 23.7, p < 0.05), and larger discordance of twin weight (14.7 ± 10.6% vs 11.7 ± 8.4%, p > 0.05). Additionally, Apgar score ≤ 7 at one minute was found in 40% of MC, as compared to 29.8% of DC (p < 0.05), though the rate of Apgar score ≤ 7 at five minutes was similar in the two subgroups (Table 1).

With regards to the drug treatments, there were no statistically significant differences between the two groups, except for a greater use of antenatal corticosteroid therapy in the MC subgroup (38.2% vs 26.2%, p < 0.05).

Table 2. — Comparison between spontaneous and ART twin pregnancies.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SC group (n=82)</th>
<th>ART group (n=56)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (years) (mean ± SD)</td>
<td>33±5</td>
<td>32.4±5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>48 (58.5%)</td>
<td>53 (94.6%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>≥1</td>
<td>34 (41.5%)</td>
<td>3 (5.4%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Ante-partum stay (days) (mean ± SD)</td>
<td>4 ± 9</td>
<td>4 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Post-partum stay (days) (mean ± SD)</td>
<td>4 ± 2</td>
<td>5 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous delivery</td>
<td>4 (5%)</td>
<td>1 (1.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>78 (95%)</td>
<td>55 (98.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Neonatal outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight (grams) (mean ± SD)</td>
<td>2,176 ± 523</td>
<td>2,186 ± 456</td>
<td>NS</td>
</tr>
<tr>
<td>Percentile</td>
<td>47 ± 24</td>
<td>45.5 ± 23</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar 1 minute ≤7</td>
<td>30%</td>
<td>30.5%</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar 5 minutes ≤7</td>
<td>3.6%</td>
<td>5.3%</td>
<td>NS</td>
</tr>
<tr>
<td>IUD</td>
<td>3.7%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPROM</td>
<td>12.2%</td>
<td>16.1%</td>
<td>NS</td>
</tr>
<tr>
<td>Threatened preterm delivery</td>
<td>29.3%</td>
<td>37.5%</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery &lt; 37 weeks</td>
<td>61%</td>
<td>76.8%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Abnormal Placenta</td>
<td>1.2%</td>
<td>1.8%</td>
<td>NS</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>3.6%</td>
<td>1.4%</td>
<td>NS</td>
</tr>
<tr>
<td>Polyhydramnios</td>
<td>1.2%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>8.5%</td>
<td>3.6%</td>
<td>NS</td>
</tr>
<tr>
<td>IUGR</td>
<td>11%</td>
<td>13%</td>
<td>NS</td>
</tr>
<tr>
<td>Selective IUGR</td>
<td>4.9%</td>
<td>7.1%</td>
<td>NS</td>
</tr>
<tr>
<td>PE/HELLP</td>
<td>6.1%</td>
<td>10.7%</td>
<td>NS</td>
</tr>
<tr>
<td>PIH</td>
<td>12.2%</td>
<td>14.3%</td>
<td>NS</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>11%</td>
<td>12.5%</td>
<td>NS</td>
</tr>
<tr>
<td>GDM</td>
<td>1.2%</td>
<td>3.6%</td>
<td>NS</td>
</tr>
<tr>
<td>TTTS</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
</tbody>
</table>

DC twin pregnancies conceived by ART versus SC

The authors compared 56 DC pregnancies conceived by ART (IVF-ET/ICSI) and 82 DC pregnancies conceived spontaneously. Maternal age, duration of ante and post-partum stay, type of delivery, birth weight, and short-term neonatal outcome were not significantly different between subgroups. Parity in the ART subgroup was 0.05 ± 0.22, and 0.41 ± 0.49 in the SC group (p < 0.05).

The incidence of abnormal placentation, placental abruption, IUD, PIH, PE, HELLP syndrome, cholestasis, GDM, and IUGR was not significantly different between the two subgroups.

The ART pregnancies showed a higher incidence of preterm delivery before 37 weeks (76.8% vs 61.0%, p < 0.05) (Table 2).

Additionally, women in the ART subgroup received more frequently drug treatments, in particular low-molecular-weight heparin LMWH (50.0% vs 22.0%, p = 0.001), cardioaspirin (23.2% vs 6.1%, p = 0.005), and prednisone (14.3% vs 0%; p = 0.001), as well as the administration of progesterone (30.4% vs 12.2%, p = 0.01). Similarly, the antenatal corticosteroid therapy with betamethasone was administered more frequently in the ART subgroup (35.7% vs 19.5%, p = 0.01).

Discussion

Twin pregnancy is burdened by a higher risk of adverse outcomes compared with singleton. This issue is becoming more frequent in clinical practice, and subsequently topical in clinical literature, due to the dramatic increase of the incidence of multiple births in the developed countries over the past decades.

Most studies showed a higher incidence of preterm births and adverse neonatal outcomes in MC pregnancies, possibly related to TTTS and selective IUGR [5, 7]. According to Acosta-Rojas et al., the adverse perinatal outcomes of MC twins appear to be associated with selective IUGR [6].

In accordance with literature data, the present study found that in the MC subgroup GA at admission was one week earlier than DC, likely related to the higher incidence of pPROM, preterm births, IUGR, and TTTS. However, the present data confirmed the greater incidence of selective IUGR, though not statistically significant in MC pregnancies. Additionally, the present authors found a higher incidence of adverse short-term neonatal outcomes in the MC subgroup, as fairly expected.

Literature data concerning the maternal complications are controversial [7, 12, 13]. The present study did not disclose any difference between MC and DC twin pregnancies. Hypertensive disorders were more frequently associated to DC pregnancy, though this evidence was not statistically significant. Nonetheless, a limitation of the present study may be represented by the low number of MC pregnancies.
The present study further investigated the impact of ART on the outcome of DC pregnancies. ART has been related to a higher risk of maternal-fetal complications and preterm birth in some studies, that compare the outcomes of singleton pregnancies conceived by ART with those conceived spontaneously [14-17]. On the contrary, several studies investigating the outcome of twin pregnancies conceived by ART have produced conflicting results [14, 18-27].

Interestingly, a recent meta-analysis of perinatal risks in twins, which selected studies that matched or controlled for maternal age and others factors, showed that ART twins had an increased risk of preterm birth and low birth weight compared to SC twins [28]. However, this meta-analysis was not adjusted for chorionicity, albeit this is an important prognostic factor in both ART and SC twin pregnancies.

In the present study, the authors restricted their analysis of the impact of ART to the DC subgroup in order to avoid the bias of monochorionicity. Other studies carried out this analysis after controlling for chorionicity or zygosity but their results are inconsistent [24, 29]. Furthermore, maternal age is supposed to be an additional variable eventually affecting the outcome of ART twin pregnancies. In fact, women delivering after ART are often older than the average population of pregnant women and almost always nulliparae, and this fact has often been used to explain the adverse obstetric and perinatal outcomes observed in these patients.

In the present study, the authors found lower parity in the ART women, as fairly expected. Interestingly, the maternal age (ART versus SC) was similar in the two subgroups, thus reducing the influence of this variable. Thus, the present findings may be considered controlled for chorionicity, due to methodological limitations, and for maternal age, by chance. Actually, the outcomes of ART twin pregnancies were generally comparable with SC twin pregnancies, except for a higher incidence of preterm delivery and a related use of antenatal corticosteroid therapy.

In the literature, the increased risk of preterm birth has been suggested to be secondary to higher concentrations of relaxin throughout gestation following gonadotropin stimulation [30]. Another factor that may contribute to higher rates of preterm birth is the increased rate of obstetric intervention, since ART birth is often the first birth after a history of infertility, so that both the physician and mother may be more worried about delivery than in SC pregnancies [31]. With regards to maternal complications, most studies do not find any statistically significant difference between ART and SC twin pregnancies [15, 19, 23, 25]. A higher prevalence of GDM in the ART group, possibly explained by the high rate in ART group of women suffering from polycystic ovary syndrome, which is associated with insulin resistance, has been anecdotally reported [21]. Additionally, a higher incidence of pPROM in the ART group, eventually affecting a higher rate of prematurity, has been suggested [20]. Higher rates of placenta previa and placental abruption occur more frequently in the ART group. This could be a consequence of embryo transfer through the vagina and cervix, as well as defective uteroplacental interactions related to female fertility problems [14, 27]. Finally, a higher percentage PIH has been hypothesized to be eventually explained by the different initiation of the chorion formation while the embryo is in vitro, leading to an abnormal placentalion in both location and function [18].

A recent review about ART twin pregnancies reported increased obstetrical risks only in women with a pre-existing medical condition such as hypertensive disorders or diabetes [32], albeit most of these risks can be avoided with single-embryo transfer or with two single-embryo transfers resulting in two singleton pregnancies [33].

In the present study, the incidence of maternal complications was not significantly different between SC and ART subgroups. It is worth to note that in the ART subgroup there was a greater use of drugs, in particular LMWH, cardioaspirin, and prednisone. The impact of this medical therapy on preventing maternal complications, in particular the hypertensive disorders, is far from being understood.

Conclusion

The present study did not disclose any significant difference in the short-term neonatal outcomes between SC and ART DC twins. MC twin pregnancy was associated with higher risk of adverse outcomes compared with DC pregnancy, as previously reported. In particular, a higher incidence of TTTS, IUGR, preterm birth, a related more frequent use of antenatal corticosteroid therapy, and worse short-term neonatal outcome were noticed. Moreover, the present analysis of DC twin pregnancies suggests that ART is possibly related to a higher incidence of preterm delivery and a related use of antenatal corticosteroid therapy if compared to SC twin pregnancies.

Further studies are advocated to confirm the present findings and investigate possible difference in neonatal morbidity (incidence of respiratory distress syndrome, intraventricular haemorrhage, necrotizing enterocolitis, etc).

Acknowledgements

In memory of Professor M. M. Anceschi.

References

The impact of chorionicity and mode of conception on maternal-neonatal outcome in twin pregnancies


Comparison of mechanical artificial shrinkage methods in mouse blastocyst vitrification

J.K. Joo1, J.E. Jeong1, S.C. Kim1, C.W. Kim3, G.R. Ko2, K.S. Lee1

1 Department of Obstetrics and Gynecology, Medical Research Institute, Pusan National University, School of Medicine, Busan
2 Infertility clinic, Pusan National University Hospital, Busan
3 Department of Obstetrics & Gynecology, School of Medicine, Sungkyunkwan University School of Medicine, Samsung Changwon Hospital, Changwon (Korea)

Summary
Purpose of investigation: This study was designed to determine which mechanical artificial shrinkage (AS) method, conducted by puncture, pipetting, or aspiration, was effective in increasing the re-expansion rate of mouse blastocysts. Materials and Methods: In each group, 30 mouse blastocysts were used. Before vitrification, the blastocoelic cavity was collapsed by puncture with a micro-needle, pipetting with a micro-glass pipette, and direct aspiration with an ICSI pipette. After thawing, the re-expansion rate of blastocysts was examined for each AS method. Re-expansion rate was checked at three, five, and seven hours after thawing. Results: The number of re-expanded mouse blastocysts at five hours after thawing was 12 in the puncture with a micro-needle group, 11 in the pipetting with a micro-glass pipette group, and 24 in the direct aspiration with an ICSI pipette group. The cumulative number of re-expanded mouse blastocysts between five and seven hours after thawing (p = 0.001 and 0.021, respectively). Conclusions: Direct aspiration with an ICSI pipette resulted in a higher re-expansion rate than the puncture and pipetting methods. It can be considered that the direct aspiration method is more convenient and simpler than the other two methods.

Key words: Artificial shrinkage; Blastocyst; Assisted hatching.

Introduction
Due to the transfer of frozen-thawed embryos, the outcomes of IVF programs have improved [1, 2]. Frozen-thawed blastocyst transfer is performed more widely because of the increase in surplus embryos due to development of culture system and cryopreservation techniques [3].

During the vitrification process, the intracellular water is converted into ice crystals, and this is a major cause of embryo damage and lower survival rate [4]. Because the amount of intracellular water in the blastocyst is greater than that in the cleavage stage embryos, dehydration of intracellular water and influx of cryoprotectant take more time in the blastocyst. During the vitrification process, this factor could cause damage to blastocysts.

To overcome this problem, artificial shrinkage (AS) was introduced. Several studies reported the results of artificial shrinkage of blastocysts using various methods like glass micro-needle, a 29-gauge needle, micro-pipetting with a hand-drawn Pasteur pipette, laser pulse, and osmotic shock [5-8]. There are some reports suggesting that laser pulse is better than the other methods, but other studies reported opposite results. Also, among the mechanical methods, the best AS method is still unclear [5].

In this article, the authors compared survival and re-expansion rate of mouse blastocysts after artificial shrinkage and vitrification with three different mechanical artificial shrinkage methods, which were punctured with a micro-needle, pipetting with a hand-drawn glass pipette, and direct aspiration with an ICSI pipette.

Materials and Methods
Preparation of mouse blastocysts
Mouse two-cell embryos were collected from hyperstimulated female mice and cultured in G1.1 and G2.2 to blastocysts stage.

Preparation of equilibration and vitrification solutions
The solutions for equilibration, vitrification, and thawing were prepared using Dulbecco’s phosphate-buffered saline (PB1) plus 20% synthetic serum substitute (SSS). Equilibration solution consisted of EBS1 (G10) and EBS2 (G10E20). EBS1 and EBS2 contained 10% glycerol and 10% glycerol + 20% ethylene glycol, respectively. Vitrification solution (VS) was composed of 25% glycerol + 25% ethylene glycol in 20% SSS + PB8.

Artificial shrinkage of expanding blastocysts
Before the vitrification procedure, AS of expanding blastocysts was performed in two equilibration solutions. In this study, we used three different AS methods.
Figure 1. — Photographs of artificial shrinkage of a mouse blastocyst by insertion of a micro-needle. (A) immobilization of a mouse blastocyst with the inner cell mass at the six o’clock (B) insertion of a needle inside the blastocoele (C) shrinkage after one minute (x200) artificial shrinkage using the micro-needle.

Figure 2. — Photographs of artificial shrinkage of a mouse blastocyst by pipetting with 100 um (O.D) glass pipette connected to the injector of a micro-manipulator. (A) Before artificial shrinkage. (B) Start of pipetting. (C) A completely pipetted mouse blastocyst. (D) Shrunken mouse blastocyst (x200).
Comparison of mechanical artificial shrinkage methods in mouse blastocyst vitrification

1) Puncture with a micro-needle (group A)

The puncture needle was prepared by using a puller. It was inserted into the blastocyst and it created two holes in the blastocyst. The technique of shrinkage using micro-needle puncture has been described previously [5]. The expanding blastocyst was held with a holding pipette and the inner cell mass (ICM) was placed at the six or 12 o’clock position, and a glass micro-needle was pushed through the cellular junction of the trophectoderm into the blastocoelic cavity until it shrunk (Figure 1). After removing the micro-needle, the authors observed contraction of the blastocoel within a few minutes.

2) Pipetting with a hand-drawn glass pipette (group B)

Pipetting of the expanding blastocyst was conducted using a glass pipette with slightly smaller in diameter than the expanding blastocyst. The pipettes were manually made from a Borosilicate micro-glass pipette hand-drawn using a microforge. The inner diameter of the pipette was 100 um. The pipette was connected with the pipette holder of a micromanipulator. Pipetting was repeated until the blastocoel collapsed completely (Figure 2).

3) Direct aspiration of the blastocoelic fluid with an ICSI pipette (group C)

In the same manner as in the ICSI procedure, the blastocyst was fixed with a holding pipette after the ICM part in the expanding mouse blastocyst turning to a six or 12 o’clock. Then, the ICSI pipette was inserted into the blastocoelic cavity and the blastocoelic fluid was aspirated (Figure 3).

Continuous culture, exposure to the vitrification solution, and vitrification

First, to compare the effects of AS methods, blastocysts were cultured immediately after AS in each group and the re-expansion rate was checked. As the next step, to observe the effects of vitrification solution on artificially shrunken blastocysts, blastocysts were shrunken using each AS method and exposed to the VS solution. Immediately after exposure to the VS solution, blastocysts were cultured for seven hours.

The authors also checked the re-expansion rate of frozen-warmed blastocysts. After blastocoel contraction, the blastocysts were equilibrated in the equilibration solution for another two minutes before exposure to the vitrification solution. The blastocysts were then incubated in the vitrification solution and loaded on the tip of the capped-pulled straw within 30 seconds. Then, the straw was immediately plunged into liquid nitrogen.

Thawing of blastocysts and observation of re-expansion rate

Re-expansion was defined as the full expanding state of the blastocyst. The proportion of re-expanding blastocysts at three, five, and seven hours after thawing was observed in each group.

Statistical analysis

The data obtained were examined for differences using Student’s t-test and Fisher’s exact probability test as appropriate. Results are expressed as mean ± SD.

Results

The procedures used in this study are described in Figures 1, 2, and 3. The conduction time was less than three minutes for all three methods, respectively. For adequate shrinkage, puncture was performed at least two times and pipetting was performed three times. Direct aspiration was
Table 1. — Cumulative number of re-expanded mouse blastocysts after artificial shrinkage with three different mechanical methods.

<table>
<thead>
<tr>
<th>No. of re-expanded mouse blastocysts according to the passage of time</th>
<th>Methods of artificial shrinkage</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A (Micro needle, n=30)</td>
<td>Group B (Glass pipette, n=30)</td>
</tr>
<tr>
<td>3 h (%)</td>
<td>3 (10.0)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>5 h (%)</td>
<td>16 (53.3)</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>7 h (%)</td>
<td>23 (76.7)</td>
<td>24 (80.0)</td>
</tr>
</tbody>
</table>

Table 2. — Cumulative number of re-expanded mouse blastocysts exposed to the vitrification solution after artificial shrinkage with three different mechanical methods.

<table>
<thead>
<tr>
<th>No. of re-expanded mouse blastocysts according to the passage of time</th>
<th>Methods of artificial shrinkage</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A (Micro needle, n=30)</td>
<td>Group B (Glass pipette, n=30)</td>
</tr>
<tr>
<td>3 h (%)</td>
<td>2 (6.7)</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>5 h (%)</td>
<td>14 (46.7)</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>7 h (%)</td>
<td>21 (70.0)</td>
<td>23 (76.7)</td>
</tr>
</tbody>
</table>

Table 3. — Cumulative number of re-expanded mouse blastocysts after vitrification and warming following artificial shrinkage with three different mechanical methods.

<table>
<thead>
<tr>
<th>No. of re-expanded mouse blastocysts according to the passage of time</th>
<th>Methods of artificial shrinkage</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A (Micro needle, n=30)</td>
<td>Group B (Glass pipette, n=30)</td>
</tr>
<tr>
<td>3 h (%)</td>
<td>2 (6.7)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>5 h (%)</td>
<td>12 (40.0)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>7 h (%)</td>
<td>20 (66.7)</td>
<td>20 (66.7)</td>
</tr>
</tbody>
</table>

Discussion

In slow freezing of blastocysts, the survival rate was very low [9]. The reasons for this are damage to the trophoblast caused in the process of dehydration by the freezing solution and ice crystal formation due to insufficient dehydration [10-12].

For overcoming these problems, AS of blastocysts was introduced. Many studies have reported the beneficial effects of AS of blastocysts [5-7]. Mukaida et al. reported a high survival rate (97.2%) and pregnancy rate (60.2%) in warmed-vitrified blastocyst transfer cycles using AS before vitrification [4].

Several different AS methods were introduced, such as micro-needle stimuli [4, 5], 29-G needle puncture [6], laser pulse [4], and pipetting [7]. Among these methods, laser pulse is used more widely, but the laser equipment is quite expensive and is not always possible to provide this facility in all IVF clinics. AS methods using a needle or pipette however do not need any special equipment and the biologist in the IVF labs familiar with the procedure. Considering the economic aspect and accessibility, these methods are very feasible.

In this study, the re-expansion rate in the direct aspiration with an ICSI pipette group was higher than that in the other two groups. Many factors can be considered to be responsible for this result such as human error and mechanical damage. Technical errors can occur due to the complexity of methods. During all of the AS procedures, a micromanipulator was used. This made delicate handling of the blastocysts possible and the probability of occurrence of human errors was controlled well in all of the three AS methods.

Lesser mechanical damage might be the main cause for the good result in the direct aspiration group. In the puncture method, puncture was performed at least two times. Also, in the pipetting method, pipetting was performed two to three times to cause complete shrinkage. Repeat procedures might cause trophoblast damage and influence the survival rate and re-expansion rate.
Direct aspiration of the blastocoelic fluid might have some other benefits. It is a familiar method for those who conduct the ICSI procedure. A relatively short procedure time can be helpful for survival of the embryo and for the laboratory technician. It could result in less human errors.

These methods can be applied to the human embryo, and actually in the present clinics, this procedure was used several years ago [13]. However, the present authors have no comparative data in human IVF.

The results in humans could be different from those in mouse blastocysts. However, the aspiration method is relatively easy and simple, but a learning curve is needed to achieve consistent results in the human IVF program. This study showed statistically better outcomes in the aspiration group and it may be valuable for further research.

Considering the economic aspect and accessibility, mechanical AS is considered to be an efficient method for frozen-warmed blastocyst transfer. Especially, the present authors think that direct aspiration of the blastocoelic fluid with an ICSI pipette is a very simple and cost-effective method.

References


Address reprint requests to:
KYU-SUP LEE, M.D.
Department of Obstetrics and Gynecology
Pusan National University, School of Medicine
Gu-Deok-Ro 305, Seo-Gu
Busan (Republic of Korea)
e-mail: kuslee@pusan.ac.kr
The possible role of serum leptin in preeclampsia

Y. Doster¹, B. Cetinkaya Demir², M.A. Atalay², E.E. Durusoy², S. Kucukkomurcu²

¹Department of Obstetrics and Gynecology, Medipol University School of Medicine, Istanbul
²Department of Obstetrics and Gynecology, Uludag University School of Medicine, Bursa (Turkey)

Summary

Background: It is theorized that adipokines play a critical role in the pathophysiology of preeclampsia, particularly with their pro-inflammatory and inflammatory features. Aim: To investigate serum leptin levels in pregnancies complicated with preeclampsia and severe preeclampsia. Materials and Methods: Maternal serum leptin levels were analyzed by solid phase enzyme amplified sensitivity immunoassay (EASIA) method in 23 patients with mild preeclampsia, 29 patients with severe preeclampsia, and 28 healthy pregnant controls. Results: Mean serum leptin levels did not differ statistically between patients with mild preeclampsia, severe preeclampsia, and the controls (10.77 ng/ml, 13.40 ng/ml, and 8.43 ng/ml, respectively). Also, there was no relationship between serum leptin levels and the gestational ages of the participants. Discussion: Serum leptin levels are not associated with preeclampsia. Leptin measurements are not affected with the gestational age. The role of leptin in the pathophysiology of preeclampsia should be evaluated cautiously.

Key words: Preeclampsia; Severe preeclampsia; Adipokine; Leptin.

Introduction

At the present time, the pathophysiology of preeclampsia is still obscure. Recent studies suggest that adipokines may play an important role during pregnancy, and therefore, may have a role in the pathologic basis of this rigorous disorder [1-3]. Principally, adipokines that are secreted from the adipose tissue are known to regulate the secretion of insulin [4]. Thus, they control the metabolism of the organism. Additionally leptin, one of the adipokines, has functional and structural similarities to interleukin-6 (IL-6) family of cytokines [5, 6]. It is introduced as a pro-inflammatory cytokine. Leptin is also a pro-angiogenic and mitogenic factor, the actions of which are reinforced through crosstalk with IL-1 family of cytokines. It is well-established that leptin is involved in the regulation of the inflammatory response [6-8]. This may explain its potential participation in development of preeclampsia [6-8]. The placenta is one of the major sources for leptin [9]. There are evidences that the production of placental leptin is reinforced with hypoxia [10, 11]. Leptin is thought to ensure adequate nutrient transfer for placental–fetal use [12]. Regulation of the leptin mechanism is said to be discrete in pregnancies in which fetal growth and development is pathologic, compared to physiologic regulations in normal pregnancies [13-15]. Placental insufficiency is associated with a significant increase in placental leptin production, suggesting that leptin may be an index of fetal stress and placental dysfunction [16], but it is not evidently concluded that serum leptin levels increase in patients with preeclampsia. Therefore, in this study, the authors sought to determine serum leptin levels in patients with preeclampsia in accordance with the severity of the disorder.

Materials and Methods

Participants

Fifty-two preeclamptic women [23 patients with mild preeclampsia (MPE) and 29 patients with severe preeclampsia (SPE)] and 28 normotensive pregnant controls were recruited from patients managed at the antenatal clinics of the Obstetrics and Gynecology Department of Uludag University between April 2009 and May 2011. All participants were Caucasian third-trimester singleton pregnancies with average socioeconomic status. The attendees of the control group were followed up with blood pressure measurements beyond the sixth week after delivery to ensure that they did not develop chronic hypertension.

Definition of exposure and study population

Preeclampsia was defined as demonstration of systolic blood pressure measurement above 140 mm Hg and diastolic blood pressure reading above 90 mm Hg on at least two different occasions more than six hours apart in a previously normotensive women after 20th gestational weeks at her pregnancy, in association with proteinuria above 300 mg/l in a 24-hour urine collection [17]. SPE was defined as presence of one of the following criteria: systolic blood pressure measurement above 160 mm Hg or diastolic blood pressure measurement above 110 mm Hg on two occasions at least six hours apart, more than five grams urinary protein excretion in 24 hours, less than 500 ml of urinary discharge in 24 hours, increased serum creatinine levels above 1.2 mg/dl, presence of microangiopathy demonstrated with thrombocyte counting lower than <100,000/mm³ or increased lactate dehydrogenase more than 600 u/l, presence of cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper quadrant pain [17]. Body mass index...
Table 1. — Patient demographics.

<table>
<thead>
<tr>
<th></th>
<th>MPE group (n=23)</th>
<th>SPE group (n=29)</th>
<th>Control group (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 (19-33)</td>
<td>26 (19-43)</td>
<td>30 (22-39)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td>1 (1-4)</td>
<td>1 (1-5)</td>
<td>2 (1-5)</td>
<td>0.111*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0-3)</td>
<td>0 (0-4)</td>
<td>0 (0-2)</td>
<td>0.263*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gestational age (weeks)</td>
<td>36 (28-38)</td>
<td>36 (28-40)</td>
<td>39 (36-41)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.84 (16.6-44.4)</td>
<td>28.76 (22.76-41.45)</td>
<td>28.71 (21.83-45.17)</td>
<td>0.442</td>
</tr>
</tbody>
</table>

*Statistical significance of the difference between control and MPE groups.

Table 2. — Mean serum leptin levels of the study participants.

<table>
<thead>
<tr>
<th></th>
<th>MPE group (n=23)</th>
<th>SPE group (n=29)</th>
<th>Control group (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>10.77 (1.55-49.33)</td>
<td>13.4 (2.07-46.35)</td>
<td>8.43 (2.36-43.48)</td>
<td>0.155</td>
</tr>
</tbody>
</table>

MPE: mild preeclampsia; SPE: severe preeclampsia

Results

The clinical characteristics of the subgroups are summarized in Table 1. The median ages of the patients with MPE, SPE and control participants were 26 years (19-33), 26 years (19-43), and 30 years (22-39), respectively. There was no statistical significance with respect to the ages between patients with mild and severe preeclampsia, but patients with MPE and controls, and SPEs and controls. The differences of gravidity and parity were statistically significant solely between the patients with SPE and controls. The differences between median gestational ages of the patients with MPE (36 weeks), SPE (31 weeks), and the control participants (39 weeks) were statistically significant (Table 1).

The median values of BMI in MPE, SPE, and control groups were 28.84 kg/m² (16.6–44.4), 28.76 kg/m² (22.76–41.45), and 28.71 kg/m² (21.83–45.17), respectively. None of the differences between the groups in terms of BMI was statistically significant (Table 1).

Mean serum leptin level was 8.43 ng/ml (2.36–43.48) in control participants, 10.77 ng/ml (1.55–49.33) in patients with MPE, and 13.4 ng/ml (2.07–46.35) in patients with SPE. The differences between all groups were found statistically insignificant (Table 2).

We did not find any statistically significant relationship between serum leptin levels and systolic, diastolic, and mean arterial blood pressures within each group (Table 3). Serum leptin levels did not change between gestational weeks at which blood sampling was carried out in all patients and within study groups (Table 3, Figure 1). Furthermore, no statistical significance was found with respect to the gestational age, when serum leptin levels were adjusted for BMI, systolic blood pressure, diastolic
The possible role of serum leptin in preeclampsia

A positive linear correlation was found between serum leptin measurements and BMI when the authors evaluated all patients ($r = 0.414$) (Table 3, Figure 2). In none of the groups but in the controls, a positive linear correlation could be demonstrated between BMI and serum leptin levels when each group was analyzed separately ($r = 0.661$) (Table 3, Figure 3). Serum leptin levels and BMI were found to inversely correlate in patients with MPE and SPE, yet it did not reach statistical significance ($p = 0.120$ and $p = 0.128$, respectively).

The differences between mean values of fetal weights between patients with MPE and healthy controls, and patients with SPE and healthy controls were statistically significant ($p < 0.001$, $p < 0.001$). There was no correlation between serum leptin measurements and the fetal weights within the groups (Table 3).

Discussion

In this study, we could not establish a statistically significant difference in serum leptin levels between patients with preeclampsia and healthy pregnancies. Although the difference of gestational ages of the study participants between all three groups were statistically different, it is demonstrated that gestational ages of the participants had no influence on the serum leptin measurements. Similar to the present study, Teppa et al. [18] and Kafulafula et al. [19] demonstrated that leptin levels were not correlated with gestational age. The means of the gestational ages of the patients with preeclampsia and control participants in those studies were 36.6±0.4 and 38.2±0.3 weeks, and 34.4±8.3 and 35.5±4.6 weeks, respectively. Like the latter two, some other studies emphasized serum leptin measurements not to be effected by the gestational age in the third trimester of pregnancy [20, 21]. In pregnancy, by the application of Northern blot analysis, leptin is shown to be primarily expressed in the first-trimester chorionic villous tissue, and to a lesser degree in the third-trimester chorionic tissue, amnion cells and villous vascular endothelial cells [22, 23]. During pregnancy, circulating leptin levels show a trend towards an increase, especially during the second trimester, and a plateau during the third trimester, while after labor, they decline sharply to the non-pregnant levels [21, 24, 25]. In this study, the gestational ages that the serum samplings were conducted in all three groups were in coverage of the previously mentioned studies. In parallel to the present study, other investigators also failed to demonstrate any significant difference concerning serum leptin levels between pre-eclamptic and normotensive women during the third trimester [26]. Furthermore, Lam et al. have suggested that serum leptin levels were significantly lower in pre-eclamptic pregnant women than in women with normal pregnancies, while no differences were identified between the two groups concerning the leptin levels in cord blood [27].
Participants (r = 0.661, p < 0.001). In addition, we could not demonstrate a statistically significant relationship between BMI and serum leptin measures amongst the normal pregnancy study participants (r = 0.120 and p = 0.128, respectively). This finding was in accordance with the work of Molvarec et al. [30] and Eleuterio et al. [31], but partly opposing the studies of Kafulafula et al. [19] and Sattar et al. [33], which demonstrated a statistically significant correlation between BMI and serum leptin levels in both of the groups including patients with preeclampsia and normotensive individuals.

Insufficient endovascular invasion of the cytrophoblasts, which leads to placental hypoxia, is thought as the main/major pathophysiologic process in development of preeclampsia. Hypoxia was shown to increase production [34-37] and excretion of placental leptin [38]. The evidences from above mentioned studies address leptin as a part of fetal adaptation mechanisms against deteriorated placental perfusion, and therefore, help to explain possible alterations of serum levels of leptin of mother or the fetus. Although the differences between mean values of maternal serum leptin in all three groups did not differ significantly in the present study, the differences of mean values of fetal weights between patients with MPE and SPE (p = 0.120 and p = 0.128, respectively). This finding was an expected one because of the premature delivery of the fetuses in the group of patients with MPE and SPE. However, when we evaluated the weights of the infants in each group separately, we did not find any correlation between weight of the infant and maternal serum level of leptin. This finding was compatible with the results of Sucak et al. [39] but opposite to the results of Mise et al. [40].

Although some of the studies reported a relationship between serum concentrations of leptin and blood pressure, some others did not [41 - 43]. In this study, we could not demonstrate any statistically significant relationship between leptin and systolic BP, diastolic BP, and mean arterial BP, as well. The studies in which a relationship was reported asserted the presence of hemococoncentration, which is an important adherent of preeclampsia, as the causative factor for this relationship [44]. However, other studies could not demonstrate a correlation between hemococoncentration and increased levels of maternal leptin [18, 35]. In one of those studies, Teppa et al. measured total fraction of leptin [18]. Unbound fraction of leptin (rather than bound fraction) was found to be increased when they separated bound and unbound fractions by chromatography in that study. This finding stands against the hemococoncentration theory. Thus, there is no sufficient data demonstrating relationship of hemococoncentration and leptin levels in preeclamptic patients.

The gradually increasing production rate of leptin by the human placenta towards gestation, as well as the fluctuations observed during pregnancies complicated with preeclampsia could support the concept that leptin represents an important metabolic factor functionally linked with human pregnancy [45]. Studies investigating leptin levels in maternal serum of the patients with preeclampsia yielded different results. There are many studies that reported increased levels of leptin in preeclampsia [46 - 49], however, others declared no change [26, 50, 51] or decrement [27] in leptin levels in preeclampsia. It seems that there are several mechanisms in regulation of leptin in preeclampsia. The discrepancy in the studies could be attributed to multiple factors which affect serum levels of leptin including the influence of weight gain on pregnancy itself, and the ethnicity. Certain confounding factors such as the differences in study designs, patient selection criteria, and non-homogenous patient groups may also contribute to these inconsistent results. Further researches will provide new insights into the physiological mechanisms of leptin in the pregnant women and its possible role in complex disorders like preeclampsia.

References

Effect of pregnancy-specific stress on spontaneous preterm birth among Chinese people

X.L. Qu¹, W.J. Zhu¹, W.Q. Chen², Y.Y. Cui³, P. He³, Z.H. He³, Z.L. Wang¹
¹ The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou
² School of Public Health, Sun Yat-Sen University, Guangzhou
³ Guang Zhou Women and Children’s Medical Center, Guangzhou (China)

Summary
Background: The current evidence implicates that psychosocial stress, especially pregnancy-specific stress, is associated with the risk of spontaneous preterm birth. The aim of the present study was to determine the effect of pregnancy-specific stress on spontaneous preterm birth among Chinese people. Materials and Methods: A total of 2,189 pregnant women were enrolled and followed up until parturition from February 2011 to January 2012. Maternal pregnancy-specific stress was assessed using the revised Pregnancy Stress Rating Scale (PSRS) at third trimester in pregnancy. Socio-demographic and psychological data were collected through interviews, medical, and obstetrical examination records. Results: High levels of maternal pregnancy-specific stress during the third trimester increased risk of spontaneous preterm birth compared with the low and medium levels (adjusted risk ratios, 2.92; 95% confidence interval, 1.12 - 7.58). The first stressor from the revised PSRS includes a risk factor for the safety of infants. Conclusions: High level of pregnancy-specific stress in third trimester might predict spontaneous preterm birth.

Key words: Pregnancy-specific stress; Spontaneous preterm birth; Revised PSRS; Third trimester; Stressor; Effect of PEQ on SPB.

Introduction
According to reports, 9.6% of total births across the globe in 2005 were preterm birth that accounts for 85% in Africa and Asia [1]. Prevalence of preterm birth is similar in China, ranging from 5-15%. Preterm birth is a major contributor to neonatal morbidity and mortality in poor perinatal outcomes. Some long-term complications, particularly cardiovascular and metabolic diseases which affect premature infants, could persist in later adult life [2]. Preterm delivery becomes a significant perinatal health problem throughout the world [1], which leads to an important economic burden for the society and individual. Approximately 75% of preterm births are attributed to spontaneous preterm birth; its etiology is still however unknown.

Psychosocial stress has been observed to be responsible for preterm birth and a crucial risk factor for poor perinatal outcomes in recent years. Although the methodology to measure psychosocial stress have improved, it appears that the association between stress and adverse perinatal outcomes remains controversial. Stressors such as major life events, work stress, daily hassles, and neighborhood safety et al. were investigated by most studies, whereas pregnancy-specific stress as a potential risk factor for spontaneous preterm birth was less studied. Pregnancy-specific stress, defined as maternal fears and worries pertaining to pregnancy [3], was indicated as a reliable predictor of preterm birth in recent years [4, 5]. Most studies paid attention to the influence of pregnancy-specific stress on preterm birth at a certain gestational period [4, 6], but few were interested in investigating the exposure time [3] and perceived impact originated from pregnancy-specific stress. Especially in China, it is scarcely regarded as a risk factor for preterm birth.

Coping was defined as “cognitive and behavioral efforts to manage demands perceived as taxing or exceeding one’s resources” [7]. Coping is a mediator, which pregnant women could use to help deal with psychological stress. As a buffer of stress, social support might have a positive effect on pregnant women. It can inspire and encourage pregnant women to take measures to alleviate psychological stress, alter bad lifestyles, and persist in regular antepartum care. It was been shown in few articles that preterm birth could occur when faced with the absence of partner support [8]. Nevertheless, most studies demonstrated that social support could decrease the incidence of preterm birth [9, 10].

Maternal psychosocial stress has been extensively examined; to the present authors’ knowledge, epidemiologic research remains inadequate in China. Hence this study was designed with the objective to investigate the effect of spontaneous pregnancy-specific stress on preterm birth in the third trimester.
Materials and Methods

Study population

A prospective study was conducted in the First Affiliated Hospital of Sun Yat-sen University and Guangzhou Women and Children’s Medical Center from March 2011 to March 2012 and 2,575 pregnant women in third trimester were enrolled to complete a self-designed questionnaire. A total of 2,189 valid questionnaires were received, 130 of 2,189 mothers delivered premature infants and comprised the case group and 2,059 with normal term pregnancy as the control group. The questionnaire was designed by reviewing literature and referred to relevant questionnaires. They were presented to the pregnant women who participated in the antenatal examination at the two hospitals. Written consent form and detailed information of the study were obtained from all the participants.

The questionnaire consisted of three parts. The first part included socio-demographic characteristics including maternal age, years of education, average monthly income, pre-pregnant body mass index (BMI), and so on. The second part included three scales, the Revised Pregnancy Stress Rating Scale (PSRS), Simplified Coping Style Questionnaire (SCSQ), and Social Support Scale. The last part included information on birth outcomes including gestational age, birth weight, and delivery mode, etc.

The exclusion criteria were as follows: in terms of previous documented impact of race on preterm birth, the present subjects were Han Chinese. The women that had miscarriage, stillbirth, abnormality, medically induced preterm, and previous history of poor pregnancy outcome (prior preterm birth, stillbirth) were excluded from the study. Age less than 20 years, not planning to deliver, and continuing prenatal care at the two hospitals were also ineligible. Mothers who became pregnant by assisted reproductive techniques were not included either. Other women were rejected for medical and obstetric-related complications (such as heart diseases, diabetes mellitus, immunological diseases, hypertensive disorder complicating pregnancy, multiple pregnancy, mental disease, and so forth).

To assess the particular contribution of pregnancy-specific on spontaneous preterm birth, it was considered vital to eliminate confounding factors. For this reason, sociodemographic and psychological factors reported to be linked to mother’s stress and spontaneous preterm birth were controlled in the present research.

The revised PSRS

The modified inventory for the assessment of maternal pregnancy-specific stress was adapted from the 30-item PSRS and was designed in 1989 by Chen et al. and adapted with a slight modification for fitting mainland Chinese [11]. The revised PSRS was used to survey maternal exposure to the stressors experienced by gravid women during third trimester (28-36 weeks). The PSRS mainly consists of factors reported to be linked to mother’s stress and spontaneous preterm birth were controlled in the present research.

SCSQ

The Chinese Edition of SCSQ was developed by Xie in 1998 to evaluate coping style [12]. The instrument included 20 items on a four-point scale ranging from 0 (never) to 3 (always). The coping styles were sorted into two categories: positive coping (from 1 to 12 items) and negative coping (from 13 to 20 items). The total scores were calculated by average dimension of positive and negative coping and sorted into low, medium-low, medium-high, and high coping by using quartiles, respectively. Higher scores represented a better coping style. Primary data showed that the SCSQ possessed an adequate reliability (α=0.89) and a good internal consistency (Cronbach’s α= 0.78) [12].

Social Support Scale

The Chinese Edition of Social Support Scale [13] was used to evaluate the degree of pregnant women who obtained support from family, friends, neighbors, and other people through living. The self-report scale consists of ten items (three item objective support, four item subjective support, and three item utilization of social support). The ten items were summed to acquire total score ranging from 0 to 14 which were sorted into low, medium-low, medium-high, and high support by using quartiles, respectively. Higher scores represented a better social support.

Statistical analysis

Epidata 3.0 software was used to build database and questionnaire entry. The Statistical Package for Social Science (SPSS) ver-
sion 16.0 software was used to obtain descriptive statistics. Test-retest stability of the revised PSRS was evaluated using Pearson correlation. Quartiles statistics were performed to describe the coping style and social support scales. Crude risk ratios (RR) and 95% confidence intervals (CI) were performed to determine the relationship of covariates with spontaneous preterm birth by means of single-factor logistic regression analysis. Chi-square analysis was used to determine the correlation between covariates and pregnancy-specific stress. Unconditional multiple logistical regression was used to adjust the confounding factors and determine the correlation between pregnancy-specific stress and spontaneous preterm birth. The confounding factors included maternal education (≤ 12 years, >12 years), monthly household income (≤ 3000 Yuan/month, 3000 - 5000 yuan/month, > 5000 Yuan/month), pre-pregnant BMI (under weight: BMI < 18.5 kg/m², normal weight: BMI 18.5 - 24.9 kg/m², overweight or obese: BMI > 25 kg/m²), social support (low, medium-low, medium-high, high), and coping style (low, medium-low, medium-high, high). A p value of < 0.05 was considered as statistical significant in the present research.

Results

Table 1 shows the descriptive characteristics, RR, and 95% CIs for preterm birth. To summarize, the mean age of the respondents was 28.7 (SD±3.5) years, while 5.8% were more than 35 years. In the study, 130 of 2,189 mothers delivered premature infants. Nearly 75% of the participants had completed senior high school. Almost one-fifth of mothers were reported to have a lower income (21.2%). A total of 1536 (70.2%) mothers had a BMI of 18.5 - 24.9 kg/m².

The results of single-factor logistic regression analysis showed that the participant age of 25-29 years was a protective factor for spontaneous preterm birth (RR = 0.52, 95% CI: 0.28-0.97, p = 0.04). Possible risk factors for spontaneous preterm birth: years of maternal education < 12 years (RR = 1.82, 95% CI: 1.25 - 2.63, p = 0.002), average monthly income less than 3,000 Yuan (RR = 2.88, 95% CI: 1.80 - 4.60, p < 0.001), low scores of social support (RR = 2.83, 95% CI: 1.65 - 4.85, p < 0.001) and low scores of coping style (RR = 1.92, 95% CI: 1.19 - 3.09, p = 0.007). Others factors were found to have obvious correlation with spontaneous preterm birth.

Table 2 shows different levels of perceived pregnancy-specific stress across demographic and psychosocial characteristics during third trimester. Pregnancy-specific stress had no correlation with socio-demographic characteristics and psychosocial factors.
The relationship between pregnancy-specific stress and spontaneous preterm birth during third trimesters with unadjusted and adjusted confounding factors are presented in Table 3. The results of multi-factors logistic regression analysis showed that participants with high level of pregnancy-specific stress were susceptible to spontaneous preterm birth in third trimester when compared with the low and medium levels (RR = 2.96, 95% CI: 1.16 - 7.60, \( p = 0.02 \)) and the low and medium categories presented no associations with spontaneous preterm birth. Although the risk ratios of spontaneous preterm birth associated with high level of pregnancy-specific stress in third trimester slightly decreased from 2.96 to 2.92, the relationship between them remained significant after adjusting possible confounding factors such as maternal years of education, average monthly income, pre-pregnant BMI, social support, and coping style (adjusted RR = 2.92, 95% CI: 1.12 - 7.58, \( p = 0.03 \)). After adjusting confounding factors, the risk ratios of spontaneous preterm birth associated with low and medium levels of pregnancy-specific stress slightly increased without significant differences. In contrast to participants with low and medium levels of pregnancy-specific stress, women with high-level stress had more than two-fold greater risks of spontaneous preterm birth.

### Discussion

According to the findings of the study, a relevance of pregnancy-specific stress and spontaneous preterm birth by using perceived impacts during third trimesters was presented. The research indicated that prenatal maternal exposure to higher levels of stress originated from pregnancy-specific stress was associated with the increasing risk of spontaneous preterm birth during pregnancy. The finding was an extension of previous studies [14, 15]. It was implicated that pregnancy-specific stress in the third trimester had an association with spontaneous preterm birth. At present, many studies accumulated on examining the association between pregnancy-specific stress and spontaneous preterm birth in the second trimester. Dole et al. [6] declared that mothers with high counts of pregnancy-related anxiety had a high risk of preterm birth in mid-pregnancy. The study conducted by Orr et al. [4] found that pregnancy-related anxiety contributed to spontaneous preterm birth at mean of 16 weeks of gestation. Few articles paid attention to the occurring of pregnancy-specific stress during the time windows for preterm birth. Roesch et al. [16] examined the effect of state anxiety, pregnancy-specific anxiety, and perceived stress on gestational length at three time points during pregnancy. Their results showed pregnancy-specific anxiety was related to shorter gestation. Similarly, the impact of a series of stressors on several birth outcomes during three trimesters in pregnancy was investigated by Lobel et al. [17]. They found that pregnancy-specific stress directly predicted preterm birth. The present findings were partially consistent with the studies.

The reasons for the inconsistent results might be as follows. First of all, the mechanisms that underlay the relationship between stress and preterm birth remained plausible and unclear. It was considered that hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system played a vital role in stress procession. The exposure time is crucial to the influence of maternal stress on preterm birth. Hobel et al. [18] proposed the increased cortisol might lead to placental, suppress fetal pituitary, and inhibit fetal growth when mothers had high levels of stress in the first trimester. Mancuso et al. [19] found that women with high corticotropin-releasing hormone (CRH) levels and high stress at 28 to 30 weeks gestation had shorter gestational age, which might predict preterm birth in mid-pregnancy. The study of Obel et al. [20] showed psychological stress in late pregnancy was related to high levels of cortisol. It was hypothesized that maternal HPA axis might contribute to preterm birth through the association with fetal placental unit. Maternal stress caused the release of cortisol, CRH, epinephrine, and norepinephrine which increased placental CRH concentrations. In turn, placental CRH further stimulated the release of the aforementioned hormones [18] and mediated the fetal stress response [21]. Inflammation, immune reaction, and vascular disorder were involved in stress processes [22]. Finally the factors interacted and might initiate parturition.

Secondly, examination of stress processes during pregnancy was sophisticated by the influence of maternal demographic and socioeconomic characteristics and mother’s behaviors. As it is well known, maternal age, education, economic status, and race et al. all might be risk factors for preterm birth across maternal stress. Stress might affect maternal behaviors including smoking, eating, and sleeping. Maternal poor behaviors such as cigarette smoking and substance use were indicated in preterm birth [23].

Thirdly, the inconsistent results partly derived from defining and measuring stress. Regarding conceptual and methodological discrepancy, it is not simple to ascertain what type of stress might trigger preterm birth and during which trimester of pregnancy. Usually, the effects of all kinds of stress (such as life events stress, chronic stress,
perceived stress, daily hassles, pregnancy-specific anxiety, et al.) on birth outcomes were investigated [24]. Thus the study on stress and preterm birth remains difficult to assess in multiple factors.

In the current research, social support and coping styles were examined as confounder factors. The present data implicated that low levels of social support and coping style might predict preterm birth. However, they did not change the relationship between pregnancy-specific stress and spontaneous preterm birth. Norbeck and Tilden [25] formulated the ‘buffer hypothesis’ in which the social support might buffer the influence of stress on birth outcomes in pregnancy. However, the buffer evidence was lacking and most studies implicated that support hardly mediated the stress and preterm birth. Several studies indicated social support might act as moderator between stress and birth outcomes [6, 26], whereas others showed that social support did not change the effects of stress on birth outcomes such as preterm birth and gestational age [9, 27]. The present result was consistent with the latter.

Previous research involving the coping strategy, stress, and adverse birth outcomes remains inconsistent [23, 28]. Two studies implicated poor coping style increased preterm birth risk [29, 30]. In contrast, the present findings indicated coping style did not modify the relationship between stress and preterm birth. There might be two reasons for the inconsistent results: the first one is that social support and coping style might play buffer or moderator role in mediating the effects of stress on birth outcomes and the second one is that racial, cultural, and ethnic factors might influence social support and coping processes. Social support and coping style are associated with maternal characteristics such as personality.

However, the limitations of the present study should be noted. Firstly, the authors only examined one multiple psychological stressor without others, such as chronic stress, daily hassles, state-trait anxiety, etc. Moreover, physiological indicators of stress such as CRH and cortisol, etc., were not examined. The evidence that stress hormones interacted with psychological stress remained deficient. Further studies should investigate the association between stress and physiological markers of stress and find a biologic pathway through what kinds of stress cause poor birth outcomes. Secondly, a crucial measurement issue is worthy of consideration. According to the strata by perceived impact weighting of stress, pregnant women at a certain stress level comprised a very small proportion, leading to limited convincing evidence. Thirdly, the revised PSRS was scarcely employed to evaluate the relationship to preterm birth in China. Further research is required to explore the factor structure of the revised PSRS. Lastly, although this was a prospective based study, stress was measured at the end of third trimester retrospectively. Hence, recall bias was still inevitable. Despite the limitations, the methodological strengths of the present research includes prospective design, controls for possible risk factors, and assesses stressors by rating perceived impact rather than counts.

In conclusion, the evidence of association between psychosocial stress and preterm birth was confirmed in the present study. Moreover, it is suggested that third trimester-specific exposure may play a crucial role in the relationship between psychosocial stress and preterm birth. In addition, an approach as intervention to decrease spontaneous preterm birth risk was provided. Future studies should be carried out to examine more stressors and explore possible biologic pathway during pregnancy.

References


Address reprint requests to:
Z.L. WANG, M.D.
The First Affiliated Hospital of Sun Yat-Sen University
No. 58, Zhongshan Second Road
Yuexiu District, Guangzhou
Guangdong Province, 510000 (China)
e-mail: zilian1224@sina.com
Chronic unremitting lower abdominal pain quickly abrogated following treatment with amphetamine

J.H. Check1,2

1 Cooper Medical School of Rowen University Department of Obstetrics and Gynecology Division of Reproductive Endocrinology and Infertility, Camden, NJ; 2 Cooper Institute for Reproductive Hormone Disorders, Mt. Laurel, NJ (USA)

Summary

Purpose: To describe a cause and treatment for chronic unremitting lower abdominal pain of long duration with unknown origin. Materials and Methods: A 50-year-old woman with 30 years of unexplained right lower quadrant pain was treated with dextroamphetamine sulfate. Results: Dramatic complete abrogation of the pain occurred within two weeks. The complete relief persisted for two years while she remains on therapy. Conclusions: Sympathetic neural hyperalgesia edema syndrome should be considered whenever there is refractory pelvic or abdominal pain.

Key words: Lower abdominal pain; Sympathetic neural hyperalgesia edema syndrome; Sympathomimetic amines; Dextroamphetamine sulfate.

Introduction

Chronic pelvic pain may be associated with the menstrual cycle and could present as dysmenorrhea, dyspareunia, at mid-cycle or pre-menstrually, or Mittelschmerz. Sometimes it is chronic lasting throughout the entire menstrual cycle but may exacerbate at mid-cycle or pre-menstrually or at least by history, associated with the menses. In all of the above circumstances endometriosis and/or adenomyosis is suspected.

Laparoscopic laser vaporization of endometriotic implants or surgical extirpation to get at deep seeded endometriosis occasionally results in long lasting relief of pain but recurrence of pain even shortly following surgery is very common [1]. Treatment with dextroamphetamine sulfate has been shown to be by far the most effective long lasting treatment of pelvic pain from “endometriosis” and the therapy is well tolerated with few or any long lasting side effects [2]. Dextroamphetamine sulfate treatment was found to be highly effective even in women failing to gain significant improvement after the laparoscopic removal of documented endometriosis [3, 4].

Some chronic pain symptoms that may be associated with endometriosis could present with extra pelvic symptoms, e.g., backache, but may still be responsive to dextroamphetamine sulfate even when herniated discs are suspected [5].

One may present with chronic introital pain which has also been found to respond dramatically to sympathomimetic amine therapy [6]. Some types of pelvic pain may not be expected to respond to sympathomimetic therapy, e.g., persistent large ovarian cyst (not an endometrioma) degenerating fibroid, and cancer of one of the pelvic structures. Sometimes gastrointestinal structures, e.g., intestines with Crohn's disease or ulcerative colitis or pseudointestinal obstruction (or pathological constipation) can be the cause of lower abdominal pain and is sometimes distinguished from a pelvic source by the presence of other bowel symptoms, e.g., diarrhea, constipation, or weight loss from malabsorption or by objective signs of bowel inflammation with colonoscopy [7-10]. Occasionally a person may present with lower abdominal pain where despite careful history and physical examination there is not a clear cut diagnosis. A case is described of chronic right lower quadrant pain lasting 30 years with an unexplained etiology that quickly responded to dextroamphetamine sulfate.

Case Report

A 20-year-old woman developed unexplained constant right lower quadrant pain. Her complete blood count and comprehensive metabolic profile were normal. The pain was not associated with her menses. A laparoscopy was basically normal but was in-
terpreted as possibly some increased vascularity consistent with "pelvic congestion syndrome". Other physicians considered the diagnosis of chronic appendicitis and recommended appendectomy. She obtained other opinions that did not think that such surgery would relieve her discomfort and she chose to reject the surgical options.

She suffered with the condition for 22 years when she decided that with the advancement of modern medicine and new diagnosti c procedures that a diagnosis and therapy could finally be accomplished. A gastroenterologist ordered a CT scan but it was negative. No new therapy was offered.

She consulted the author’s practice which was recommended by one of her friends who had long standing severe dramatic dysmenorrhea and dyspareunia, but had marked improvement following treatment with dextroamphetamine amine therapy. She was advised that though most pain syndromes, whether it be headaches, joint pain, muscular pain, pelvic pain, backache or bladder pain improves with dextroamphetamine sulfate therapy, the present author did not have a precedent for a case like hers [11]. Nevertheless, the present author was optimistic that this treatment could help her lose some of the 18 kg she gained despite dieting, improve her chronic fatigue, constipation and maybe her dry skin (she was repeatedly found to have normal thyroid studies) [10, 12, 13].

After taking just 15 mg of amphetamine salts extended release capsules, her right lower quadrant pain of 30 years duration completely disappeared within two weeks of taking the drug. All of her other symptoms also markedly improved so that she was no longer constipated and her energy markedly improved. She started at 92 kg and her weight decreased within six months to 83 kg. She has been on this therapy for two years and she remains without ever having even one episode of lower abdominal pain.

Discussion

Amphetamine therapy in the dosages prescribed is very safe, non-addicting (can be stopped suddenly at any time no matter what the duration of therapy has been without withdrawal symptoms or dependence) and is usually well tolerated (dry mouth the most frequent complaint).

The mechanism of pain and the mechanism of how this drug inhibits pain and muscle fatigue and other disorders is hypothesized to relate to one of the main functions of the sympathetic nervous system which is to inhibit cellular permeability. When sympathetic nervous system hyperfunction is present, plus some local defect in a given tissue, for some reason, chemicals and toxic factors that would normally be precluded from entering tissues is not impeded. The presence of these offending agents evokes an inflammatory response and thus causes pain. The dextroamphetamine sulfate either acts directly to replace the defective neurotransmitter or causes increased dopamine secretion which corrects the neurotransmitter defect.

From a muscle weakness standpoint it is believed that toxic elements are not precluded from entering the mitochondria and thus cause malfunction. The most vivid example of this was a wheel-chair bound woman over 25 years who was diagnosed by muscle biopsy to have a form of muscular dystrophy known as the mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) syndrome to totally regain her energy and walk and drive again [14].

The weight loss was from correcting edema which is frequently a part of this syndrome. Actually the name does not fully encompass other conditions that this treatment corrects, e.g., chronic fatigue, urticaria, and vasomotor symptoms [13, 15-18].

The consideration of the sympathetic neural hyperalgesia edema syndrome in one's differential diagnosis of pelvic or abdominal pain can save the patient the risk of ineffective surgical procedures, e.g., laparoscopy, or invasive or painful diagnostic procedures or even prevent radiation exposure as from a CT scan. The best way to diagnose the condition is simply treat with dextroamphetamine sulfate and see if the symptoms abate. This drug is not an analgesic and thus will not mask a serious condition, e.g., pending ruptured ovarian cyst, or appendicitis, or ovarian torsion.

References

Chronic unremitting lower abdominal pain quickly abrogated following treatment with amphetamine


Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com
Increased tissue permeability and sympathetic nervous system hypofunction may be the common link between dysmenorrhea, chronic pelvic pain, Mittelschmerz, and Crohn’s disease

J.H. Check\(^{1,2} \) \\ \(^{1}\) Cooper Medical School of Rowan University, Department of Obstetrics and Gynecology Division of Reproductive Endocrinology and Infertility, Camden, NJ; \(^{2}\) Cooper Institute for Reproductive Hormone Disorders, Mt. Laurel, NJ (USA)

Summary

Purpose: To determine if severe periovulatory diarrhea in a woman with Crohn’s disease for just one day may be related to increased permeability of the large bowel related to hormonal changes that occur at this time of menstrual cycle. Materials and Methods: Dextroamphetamine sulfate was given to a woman whose Crohn’s disease was markedly improved by adalimumab but who still had one day of severe diarrhea at mid-cycle. Results: She did not have any diarrhea or frequent defecation for the first two periovulatory times before she achieved pregnancy. Previously for two years there had not been one month where she did not have the severe periovulatory diarrhea. Conclusions: This case helps support the concept that the classic symptoms of Mittelschmerz in women with endometriosis may be related to periovulatory events which either cause increased permeability of an already compromised tissue, whether it be pelvic or bowel or other tissues, or these periovulatory events impair sympathetic nervous system function, which is already impaired.

Key words: Sympathetic neural hyperalgesia edema syndrome; Endometriosis; Mittelschmerz; Crohn’s disease.

Introduction

It is common belief that women who get severe pelvic pain at certain specific times in the menstrual cycle, e.g., pre-menstrual or periovulatory, probably have endometriosis [1]. It is not clear how infiltrates of menstrual tissue can cause pain at the specific times.

Removal of the implants by laparoscopic laser vaporization or excision by sharp dissection may or may not relieve the pain [2-5]. Unfortunately if surgical therapy provides palliation, it frequently is only temporary. Nevertheless, it is not clear how removal of one small implant can relieve pain.

Sometimes, the use of progesterone or estrogen suppression can relieve pain [6]. A naïve concept is that this type of therapy somehow “melts away” the endometrial implants and that relieve the pain. However, usually the pain, if relieved by these therapies, quickly returns once stopping the therapy. It is unlikely that “lesions that have melted away” will grow back immediately.

There have been several case reports and series finding one of the most effective therapies for the various types of pelvic pain, even those related to certain specific times of the menstrual cycle is dextroamphetamine sulfate [7-9]. One theory is that the pelvic pain is related to having increased cellular permeability either related to intrinsic defects on a genetic basis or acquired following some type of injury. The increased permeability allows absorption of unwanted chemicals into the tissues leading to inflammation and pain. Because of hormonal changes during the menstrual cycle, these tissues may become more permeable [8].

Another factor that may play a role in the increased tissue permeability is hypofunction of the sympathetic nervous system. One of the functions of the sympathetic nervous system is to inhibit cellular permeability [10, 11]. A variety of chronic refractory illnesses which frequently are associated with pain, but not limited to pain syndromes, respond quickly and effectively to sympathomimetic amine therapy, especially dextroamphetamine sulfate [10,11]. It is believed that this drug stimulates an increase in the neurotransmitter for the sympathetic nervous system, dopamine [11].

One of the other conditions that seems to be exacerbated by sympathetic nervous system hypofunction is Crohn’s disease which can present with pain but also with very frequent episodes of diarrhea. There have been reports of dramatic improvement of severe pain and diarrhea from Crohn’s disease that had been refractory to standard therapy including adalimumab following treatment with dextroamphetamine sulfate [12].

The case described herein supports the concept that tissue permeability and sympathetic nervous system hypofunction may link a wide variety of chronic conditions that generally
respond very well to treatment with sympathomimetic amines especially dextroamphetamine sulfate. This case, however, also supports the concept that hormonal changes during the menstrual cycle may have an effect on tissue permeability, but it may not be limited to pelvic tissues.

Case Report

A 39-year-old woman with a six-month history of primary infertility sought help. She had regular menses with 29-day intervals. She had mild cramps which started when menses began. Her past medical history was significant only in having been diagnosed with Crohn’s disease five years before. Her Crohn’s disease manifested mostly as frequent daily diarrhea without dyschezia. Her symptoms eventually showed marked improvement after treatment with adalimumab. Her diarrhea was reduced to just one day per month which occurred at the periovulatory time. It was however, quite severe with 15-16 bowel movements per day which forced her to be home bound on that day. When she had daily diarrhea prior to adalimumab treatment, this one day was always the worst day. She had been advised by her gastroenterologist that adalimumab is relatively safe during pregnancy but there is still not a vast experience. However, pregnancy sometimes can cause Crohn’s to go into remission and thus she may be able to stop the medication. At her initial consult she was explained that dextroamphetamine sulfate has been used not only to treat Crohn’s disease but can be used to improve embryo implantation by inhibiting absorption of toxic material into the endometrium and improve implantation [13]. Furthermore there are enough data to establish the safety of pharmacologic dosages of amphetamines even when taken during the first trimester [14, 15]. She decided to start amphetamines salts 15-mg extended release capsules once daily. She continued the adalimumab. Because of normal semen analysis, normal post-coital, and because she attained a mature follicle and demonstrated oocyte release, she was treated exclusively with progesterone vaginal suppositories 200 mg twice daily. She conceived on her second cycle of therapy and has delivered a live full-term baby. The patient stated that over the last two years while her Crohn’s was under reasonable control, there was not one month where she did not have the one day of severe incapacitating diarrhea at mid-cycle. Her first ovulation occurred about a month after starting the amphetamine salts and she did not have the one day of severe periovulatory diarrhea in either of the two cycles of treatment prior to her conception.

Discussion

If the present woman had severe dysmenorrhea or Mittelschmerz, most physicians would consider that she probably has endometriosis with implants on the bowel. However, she only had mild dysmenorrhea, no premenstrual component, no Mittelschmerz, and no dysmenorrhea. Thus, no symptoms of endometriosis and no evidence of it on pelvic exam or sonography. The fact that even when she had daily diarrhea before adalimumab therapy she still had this one day at mid-cycle as her worst day, supports the hypothesis that hormonal changes at mid-cycle can allow increased permeability allowing chemicals to enter the bowel and cause diarrhea. The fact that sympathomimetic amine therapy immediately corrected the problem when adalimumab failed emphasizes that relative sympathetic nervous system hypofunction compounds the problem. The fact that she developed severe diarrhea for the one periovulatory day but not premenstrual may suggest that hormonal changes at this time allow maximum tissue permeability even more than the premenstrual time. Alternatively, another possible mechanism is that periovulation hormonal changes impair sympathetic nervous system function and this in turn increases tissue permeability allowing absorption of unwanted chemicals and toxic material leading to pathophysiologic events.

References


Address reprint requests to: Jerome H. Check, M.D., Ph.D. 7447 Old York Road Melrose Park, PA 19027 (USA) e-mail: laurie@ccivf.com
Comparison of efficacy of different embolic agents on uterine leiomyoma

Y. Mu1,2, Y. Wang3, M. Li3, Y. Hu3, Z. Hao1,2

1 Medical College, Xi’an Jiaotong University, Xi’an
2 Department of Interventional Radiology, The First Affiliated Hospital of Baotou Medical College, Baotou
3 Baotou Medical College, Baotou, Inner Mongolia (China)

Summary

The aim of this study was to explore the efficiencies, postoperative side effects, and complications of uterine artery embolization (UAE) treatments for uterine leiomyoma (UL) with different embolic agents. The study included 107 patients with UL that were treated with UAE with polyvinyl alcohol (PVA group) or pingyangmycin lipiodol emulsion and silk-segment (PLES group). Six months later, the improvement rate of anaemia, the menstrual improvement rate, the incidence rate of fever, the disappearance rates of compression symptoms and abdominal symptoms in the PVA group were 93.8%, 94.7%, 22.0%, 60.0%, and 88.9%, respectively, which showed no significant difference from those in the PLES group (90.5%, 92.3%, 84.8%, 53.3%, and 81.3%, respectively). The incidence rate of fever after embolization in PVA group was significantly lower than that in PLES group ($c^2=41.958$, $p=0.000$). However, the efficacy, improvement rate of symptoms, and postoperative side effects of two groups showed no significant difference ($p>0.05$). PVA and PLES have significant efficacy for UAE treatment on patients with UL.

Key words: Hysteromyoma; Embolization; Polyvinyl alcohol; Pingyangmycin lipiodol emulsion.

Introduction

Uterine leiomyoma (UL) is the most common benign tumor occurs in female reproductive system. It easily occurs in women of childbearing age with an incidence rate of 20% to 30% in women that are more than 35 years of age and a much higher incidence rate of 51.2% to 60% in women that are 40 to 50 years of age. There are three ways for treating UL, including drug treatment, interventional therapy, and surgical treatment. Drug treatment cannot eliminate or radically cure the tumor generally and often result in recurrence or growth as the sex hormone levels recover after drug withdrawal. Surgical therapy, including hysterectomy, partial hysterectomy, myomectomy, and hysteroscopic surgery, are effective but will seriously affect the patient's quality of life if the treatment is improper. Uterine artery embolization (UAE) is rapidly developed new technology in minimally invasive treatment in the recent ten years [1]. It can maintain the uterus and can be carried out under local anesthesia, with no risk of excessive loss of blood or demand of blood transfusion, and the patients can rehabilitate quickly [1-4]. At present, UAE is a relatively safe and effective method in treatment of UL. In 1995, Ravina et al. [5] have firstly reported the application of polyvinyl alcohol (PVA) in embolization of UL. As reported by Society of Cardiovascular and Interventional Radiology (SCVIR) [6], there are 800 patients with UL worldwide receiving UAE therapy in 1998. The short-term total effective rate is 90%, and the mean reduction of myoma size is 50%. With the development of interventional treatment, the efficacy of UAE in treating UL has been accepted by the majority of gynecologists and patients [7, 8]. At present, there are many kinds of embolic agents for treating UL, with different action principles. There is no unified standard for choosing which kind of embolic agent. The efficacy, postoperative side effects and complications of different embolic agent are rarely reported.

In this study, clinically common embolic agent PVA and pingyangmycin lipiodol emulsion and silk-segment (PLES) were used for UAE of UL. The efficacy, postoperative side effects, and complications of two embolic agents were compared. The objective is to provide an experimental basis for clinical application of these embolic agents.

Materials and Methods

General information

The study enrolled 107 patients with UL from January 2009 to June 2011, aged from 27 to 50 years. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Baotou Medical College. Written informed consent was obtained from all participants. There were 52 cases of multiple myoma and 55 cases of single myoma, with a tumor size of $2.1 \times 2.2 \times 2.0$ cm to $12.0 \times 11 \times 13$ cm. There were 95 patients accompanied with various symptoms, in which 45 patients had profuse menstruation or menstrual extension, 25 patients had frequent micturition, urgent micturition or constipation.

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Citation:


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Table 1. — General data of the subjects (x ± s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years)</th>
<th>Myoma size (cm³)</th>
<th>Location of myoma</th>
<th>Number of myomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Submucous myoma</td>
<td>Subserous myoma</td>
<td>Intramural myoma</td>
<td>Multiple</td>
</tr>
<tr>
<td>PVA (n=41)</td>
<td>41.4±4.8</td>
<td>91604.8±59372.3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>PLES (n=66)</td>
<td>40.3±5.7</td>
<td>75677.3±68748.4</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>t / χ²</td>
<td>t=1.029</td>
<td>t=1.226</td>
<td>χ²=1.168</td>
<td>χ² = 0.183</td>
</tr>
<tr>
<td>p</td>
<td>0.306</td>
<td>0.223</td>
<td>0.558</td>
<td>0.669</td>
</tr>
</tbody>
</table>

PVA: polyvinyl alcohol; PLES: pingyangmycin lipiodol emulsion and silk-segment.

*Sum of diameters of multiple myomas, which was converted into diameters of single myoma [11]; hysteroymoma volume was calculated with the formula of 4πabc/3cm³ where a, b, c represent the three-dimensional diameters of myomas [12].

and other compression symptoms, and 25 cases with hypogastralgia. Among the 45 cases with profuse menstruation or menstrual extension, 37 cases were anaemic with hemoglobin in 90–129 g/L (n=33) or 60–90 g/L (n=4). In terms of the location of the myoma, 107 cases of UL could also be classified into submucous myoma (n=9), subserous myoma (n=13), and intramural myoma (n=85). Eight patients had received a myomectomy in the last two to 14 years. The general information of patients in the two groups are listed in Table 1. There was no statistical difference of the general information between the two groups (p > 0.05).

Inclusion criteria
1) Patients with UL have symptoms such as profuse menstruation, menstrual extension, anemia, frequent micturition, urgent micturition, hypogastralgia, and other symptoms. 2) Patients with UL revealing asymptomatic but requesting for therapy. 3) Patients with UL before menopause, married, and without contraindications for artery embolization. 4) Patients with UL voluntarily agreed to undergo UAE treatment.

Surgical methods
Uterine artery embolization was carried out about one week after the end of menstruation of each patient. In PVA group, 100-300 mg of 355-550 μm PVA was used [9]. In PLES group, PLES was used. According to leiomyoma size and richness of blood supply, the dosage of lipiodol was determined as 8-20 ml (average 12 ml). The dosage of pingyangmycin was 8-16 mg (average 14 mg). A 7-0 silk was cut into one-mm segment for use. Using Seldinger technique, 4-F multipurpose catheter and super-smooth guidewire were intubated through unilateral femoral artery and punctured the contralateral uterine artery for ultraselection of intubation. Circutous uterine artery or disorderly blood vessel shadows could be observed in the tumor by angiography. Embolic agents was injected into uterine artery under fluoroscopy until the blood flow slowed down and disappeared. Then guidewire-looping technique was used for contralateral UAE, and the same embolization was carried out under angiography. Finally, pelvic arteriography was performed again. Nonvisualization of bilateral uterine artery indicated complete embolization.

At one, three, and six months after the embolization, each patient underwent B-ultrasound and gynecological examinations and the anaemic patients had their hemoglobin reassessed at for to six days after menstruation.

Evaluation of therapeutic efficiency
Evaluation of therapeutic efficiency [10] was mainly based on the clinical symptoms and the variation of the myoma size. At six months after embolization, the reduction of myoma volume was more than 50% and menstrual cycle and flow recovery could be considered as marked effectiveness; the reduction of myoma volume was 20%-50% and the menstrual cycle and flow that were close to the normal level could be considered as effective, and the myoma volume reduction less than 20% and the menstrual cycle and flow that did not change significantly were considered as invalid.

Results
Clinical efficacy
Improvement of symptoms: Patients were followed up for one to 30 months with a mean of six months and there were 88 cases followed up for more than six months. Before embolization, the mean hemoglobin concentration in patients with mild to moderate anemia (n=37) was 101.2 g/L in the PVA group and 99.8 g/L in the PLES group. However, at three months after embolization, the mean hemoglobin concentrations were restored to 123.5 g/L and 121 g/L, respectively. The anemic improvement rates were 93.8% (15/16) and 90.5% (19/21) in the PVA and PLES groups, respectively, showing no statistical difference between each other (c²=0.000, p = 1.000) (Table 2).

Reduction of myoma size: Before and after embolization, the three-dimensional diameters of UL were measured using B-ultrasound imaging and then were compared between the two groups. The response rate of PVA was 97.5% (40/41) in the PVA group, showing no statistical difference from that of PLES 97.0% (64/66); c²=0.000, p = 1.000 (Table 3).

Postoperative side effects
Emboli syndrome presents nausea, vomiting, fever and abdominal pain after embolization. Twenty-one patients in the PVA group presented the emboli syndrome,
accounting for 51.2%, which was statistically significant from that of the PLES group (84.8%, $c^2=14.175$, $p=0.000$) (Table 4).

There was no patient complicated with ectopic embolization or sepsis or other serious complications postoperatively.

**Follow-up**

Eighty-eight cases were followed up for more than six months and the longest follow-up period was 30 months. No recurrence was observed in the embolized myomas during the follow-up period. New myomas were found at 20 and 28 months after embolization in two patients with multiple myomata, and one of them had underwent myomectomy 16 years ago.

**Discussion**

In this study, two kinds of clinically common embolic agents PVA and PLES are used in treatment of 41 and 66 cases of UL, respectively. The efficacy of embolic agent, postoperative side effect and complication between the two groups are compared. Results show that, UAE with PVA and PLES have obvious efficacy in treatment of UL, with no significant difference between the two groups ($p > 0.05$), which is similar with reported results (6, 13, 14). The side effects of UAE are nausea, vomiting, and abdominal pain, and there is no significant difference between the two groups ($p > 0.05$). The incidence rate of fever in PLES group is significantly higher than in PVA group ($c^2 = 41.958$, $p = 0.000$), which is related to releasing pyrogenic substance by necrotic tissue and absorbance heat of necrotic tissue. Generally, the body temperature increases with the increase of necrotic tissue. The side effect increase.

### Table 2. — The improving rates of the two groups after embolization.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time points</th>
<th>Profuse menstruation or menstrual extension</th>
<th>Hemoglobin concentration (g/L)</th>
<th>Frequent micturition</th>
<th>Hypogastralgia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>Before embolization (cases)</td>
<td>19</td>
<td>15</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>(n=41)</td>
<td>1 month after embolization (cases)</td>
<td>14</td>
<td>10</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3 months after embolization (cases)</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6 months after embolization (cases)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Improvement rate at 6 months after embolization</td>
<td>94.7% (18/19)</td>
<td>93.8% (15/16)</td>
<td>60.0% (6/10)</td>
<td>88.9% (8/9)</td>
</tr>
<tr>
<td>PLES</td>
<td>Before embolization (cases)</td>
<td>26</td>
<td>18</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>(n=66)</td>
<td>1 month after embolization (cases)</td>
<td>20</td>
<td>12</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>3 months after embolization (cases)</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>6 months after embolization (cases)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Improvement rate at 6 months after embolization</td>
<td>92.3% (24/26)</td>
<td>90.5% (19/21)</td>
<td>53.3% (8/15)</td>
<td>81.3% (13/16)</td>
</tr>
</tbody>
</table>

*Comparison of the improvement rate of six months after embolization between PV A and PLES groups.

### Table 3. — Induction of hysteromyoma after embolization in the PV A and PLES groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time points</th>
<th>No marked induction of hysteromyoma (invalid)</th>
<th>Induction of 20%-50% (Effectiveness)</th>
<th>Induction of &gt;50% (Marked effectiveness)</th>
<th>Myoma elimination (Marked effectiveness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>1 month after embolization (cases)</td>
<td>4</td>
<td>33</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>(n=41)</td>
<td>3 month after embolization (cases)</td>
<td>3</td>
<td>24</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>6 month after embolization (cases (%)</td>
<td>1 (2.4%)</td>
<td>10 (24.4%)</td>
<td>28 (68.3%)</td>
<td>2(4.9%)</td>
</tr>
<tr>
<td>PLES</td>
<td>1 month after embolization (cases)</td>
<td>6</td>
<td>41</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>(n=66)</td>
<td>3 month after embolization (cases)</td>
<td>4</td>
<td>27</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6 month after embolization (cases (%)</td>
<td>2 (3.0%)</td>
<td>16 (24.2%)</td>
<td>40 (60.6%)</td>
<td>8 (12.1%)</td>
</tr>
</tbody>
</table>

*$Comparison of the induction of hysteromyoma at 6 month after embolization between PV A and PLES groups.

### Table 4. — Incidence rate of embolism syndrome after hysteromyoma embolization in the PV A and PLES groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Postoperative Nausea</th>
<th>Vomiting</th>
<th>Abdominal bearing-down pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA (n=41)</td>
<td>9 (22.0%)</td>
<td>15 (36.6%)</td>
<td>14 (34.1%)</td>
</tr>
<tr>
<td>PLES (n=66)</td>
<td>56 (84.8%)</td>
<td>32 (48.5%)</td>
<td>28 (42.4%)</td>
</tr>
</tbody>
</table>

*$Comparison of the induction of hysteromyoma at 6 month after embolization between PV A and PLES groups.
of PVA after embolization is similar with reported results [15-18]. Except for severe pain, the side effect of PLES is also similar with reported results [6, 14]. Abdominal pain is mainly caused by ischemia, injury, pain substance release, and local swelling stimulation after embolization. The degree of pain is associated with embolization degree and level. As the embolization degree is greater, it is closer to the capillary level, hence the pain is more obvious. More severe pain in PLES group may be related with the slower microsphere expansion velocity and embolization degree close capillary level.

There are many reports on UAE in treatment of UL. Goodwin et al. [7] have reported that, the symptom-improving rate of UAE on UL is 81% and the myoma shrinks off by 92% at 18 months after surgery. The present results show that, PVA and PLES have obvious short-term efficacy in treatment of UL. However, the long-term efficacy of UAE and its effect on newborn myoma should be further confirmed [12]. The main side effects of UAE are embolism syndromes (nausea, vomiting, fever, and abdominal pain), which occur within one to two days after embolization, and are associated with the uterine ischemia. They can be relieved and eliminated by symptomatic treatment. The postoperative body temperature of patients in PVA group is about 37.5°C and drops to the normal level within two days, while it is 37.8°C to 39.6°C in PLES group and drops to the normal level within five days. Six patients in the PLES group had a postoperative temperature higher than 38.8°C, which was lowered to the normal level by using one course of antibiotics.

There are also other reported complications of UAE, such as ectopic embolism, urinary retention, purulent endometritis, sepsis, abnormal vaginal bleeding and discharge of necrotic tissues, etc. [19, 20]. No ectopic embolism occurred in this study. The occurrence of urinary retention may result from severe pain in the lower abdomen after embolization, but can be relieved as the pain alleviated. Purulent endometritis may originate from preoperative intrathecal infection or the embolic time close to the menstrual period. Thus, embolization should be carried out at one to two weeks before menstruation [21]. Sepsis is resulted from the purulent endometritis that has not been timely controlled. Irregular vaginal bleeding and discharge of necrotic tissue are mainly caused by submucosal myomas. The submucosal myomas are atrophied and necrosed after embolization, and discharged in one to three months after embolization. In this study, three cases of submucosal myomas in PLES group were self-discharged, and another four cases as well as two patients in the PVA group were shed in the uterus and extracted by vaginal forceps. The present authors believe that, embolization is better for submucosal myomas, which is consistent with the literature [22, 23].

In conclusion, PVA and PLES have obvious efficacy in UAE of UL, with high safety. There is no significant difference between two materials, but the postoperative side effects in them are significantly different. After UAE using PLES, fever should be well controlled. PVA is expensive and can be chosen in practical application according to actual situation of patients.

References

Comparison of efficacy of different embolic agents on uterine leiomyoma


Address reprint requests to:
Z. HAO, M.D.
Department of Interventional Radiology,
The First Affiliated Hospital of Baotou Medical College
No. 41 Linyin Road Kundulun District
Baotou 014010 (China)
e-mail: yongxumu@126.com
Dextroamphetamine sulfate treatment eradicates long-term chronic severe headaches from temporomandibular joint syndrome - a case that emphasizes the role of the gynecologist in treating headaches in women

J.H. Check1,2
1 Cooper Medical School of Rowan University, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ; 2 Cooper Institute For Reproductive Hormonal Disorders, P.C. Mt. Laurel, NJ (USA)

Summary
Purpose: To test sympathomimetic amine therapy on another type of chronic headache syndrome - headaches from temporal mandibular joint (TMJ) syndrome. Materials and Methods: A woman with 20 years of severe daily pain from TMJ refractory to all therapies was treated with dextroamphetamine sulfate. Results: The woman showed immediate 100% relief from sympathomimetic amine treatment saving her from an expensive jaw breaking operation that was only given a slight chance of helping. Conclusions: Unfortunately most treating physicians are unaware of this defect of sympathetic nervous system hypofunction leading to the absorption of toxins, which when it involves brain tissue, leads to severe headaches. Thus, the gynecologist who is aware of this syndrome because sympathetic nervous system hypofunction is the most common cause of pelvic pain, may need to intervene in women with chronic headaches, even TMJ.

Key words: Sympathomimetic amines; Sympathetic neural hyperalgesia syndrome; Temporomandibular joint syndrome; Migraine headaches.

Introduction
Dislocation of the temporomandibular joint (TMJ) occurs when the mandibular condyle is displaced anteriorly beyond the articular surfaces and fixation in that joint, and represents 3% of all dislocated joints reported in the body [1]. Thus with TMJ, the condyle head is displaced out of the glenoid fossa but still remains within the capsule of the joint [2].

Teeth grinding and teeth clenching (bruxism) increases the wear on the cartilage lining of the TMJ and may lead to TMJ disorders. The TMJ syndrome may include any or all of the following symptoms: stiffness, headaches, ear pain, malocclusion (bite problems), clenching sounds or locked jaws. Approximately 80% of patients with TMJ disorder have the complication of headaches. Pain is caused while opening and closing the jaw [1, 2].

There is a disorder related to hypofunction of the sympathetic nervous system that is more common in women and is associated with a wide variety of chronic pain syndromes, including pelvic and bladder pain, gastrointestinal pain, musculoskeletal pain, and headaches [3, 4]. Treatment with the sympathomimetic amine dextroamphetamine sulfate usually leads to quick marked pain relief despite years of suffering and failure to respond to conventional therapy. A case is presented of a woman with long-standing chronic headaches that was believed to be related to TMJ from teeth grinding and clenching, that responded extremely well in a short time period to treatment with dextroamphetamine sulfate.

Case Report
A 41-year-old woman presented to the present reproductive endocrinology/infertility group for secondary infertility of one year duration with her new male partner. In her history it was noted that she suffered from chronic migraine headaches for over 20 years that was present every day and occurred in 60% of her waking hours. These headaches began in her first year of law school. From nervous anxiety she clenched her teeth. TMJ syndrome was confirmed by dental exam and radiological procedures and was thought to be the cause of her migraine headaches.

She advised us that she may need to delay further infertility testing for a couple months since she was scheduled for jaw surgery to try to fix the TMJ problem, to hopefully alleviate her headaches that had failed to respond to conventional medical therapy and to dental devices. She also cautioned us that she may be limited to infertility procedures covered by her insurance since the surgery performed by her doctor of dental surgery was going to be very expensive since she did not have dental insurance.

She was advised of the possibility that her headaches could respond to dextroamphetamine sulfate and that surgery could be avoided. She had such dramatic improvement with just 15 mg of...
dextroamphetamine extended release capsules that she cancelled surgery. Her headaches have for the most part completely resolved with 30 mg per day of the medication.

Discussion

Five interesting cases of chronic refractory migraines responding quickly and effectively to dextroamphetamine sulfate have been reported in Clinical and Experimental Obstetrics and Gynecology. Two cases were published in 2008 and case one involved a 33-year-old woman also presenting for secondary infertility [5]. The pain was unbearable and daily [5]. She had some of the other symptoms of the sympathetic neural hyperalgesia edema syndrome, e.g., nocturia and edema. One dose of ten mg dextroamphetamine release capsules completely removed the pain. On occasion they returned in a mild manner but with increasing the dosage to 30 mg, the headaches never returned [5].

The second case was a 45-year-old whose headaches were premenstrual and thus a pain specialist referred her to us to see if there was a hormonal connection [5]. The headaches, which could be as long as seven days premenstrual, were so intense that she was admitted to the hospital once for an overdose of narcotics. She failed to improve with luteal phase progesterone therapy or norethindrone acetate ten mg daily. After starting dextroamphetamine sulfate 20 mg per day, she reported for the first time in five years no premenstrual headaches. She has had complete relief for five years.

Sometimes to prove efficacy of a given therapy and to provide evidence that the improvement in symptoms following therapy was not merely fortuitous, one has to prove Koch's postulates, i.e., show that symptoms return with cessation of therapy and symptoms disappear again after therapy is reinitiated. Indeed one published case supports this concept and proves Koch's postulates. A 33-year-old woman with severe migraines failed to improve despite beta blockers, topirimate, and ergotamines [6]. She was presently taking 3,600 mg ibuprofen per day but still needed supplementation with oxycodone and acetaminophen but the pain only mildly improved. She came to the present reproductive endocrine practice because he was unaware of any controlled studies proving its efficacy!! She listened to him and stopped this treatment and the headaches recurred within two days. A month later, the neurologist after she was completely relieved of pain with dextroamphetamine sulfate. She was advised by the neurologist to stop the dextroamphetamine sulfate immediately because he was unaware of any controlled studies proving its efficacy!! She listened to him and stopped this treatment and the headaches recurred within two days. A month later, at the encouragement of her husband, she re-consulted us.

One more unreported cause shows dramatic efficacy of sympathomimetic amine therapy for chronic headaches by proving Koch's postulates, but also illustrates why it is important for the gynecologist to play an active role in the diagnostic testing and treating of severe migraines. A 45-year-old woman with menorrhagia came for a consult. In taking her history she revealed 25 years of migraine headaches that had failed every "standard" treatment. Despite seeking the opinion of multiple neurologists, she decided on one who at least, in her opinion, gave her the reason for them, i.e., he suspected that this was the beginning of multiple sclerosis!! However, despite careful testing so far over 25 years, she never developed multiple sclerosis.

Since the headaches occurred many years before the menstrual irregularity began we did not think there was any connection to her menstrual disorders. She responded quickly and with 100% efficacy to treatment with dextroamphetamine sulfate. She kept her appointment with her neurologist after she was completely relieved of pain with dextroamphetamine sulfate. She was advised by the neurologist to stop the dextroamphetamine sulfate immediately because he was unaware of any controlled studies proving its efficacy!! She listened to him and stopped this treatment and the headaches recurred within two days. A month later, at the encouragement of her husband, she re-consulted us. She was re-started on the amphetamine and again her headaches quickly completely disappeared and have remained so for over a year.

The frequent but relatively unknown condition that is more common in women than men by a 10:1 ratio is more known to the gynecologist because it is the most common and most remediable cause of pelvic pain [1, 2, 10, 11]. Most articles dealing with the sympathetic neural hyperalgesia edema syndrome have been published in the gynecological literature, especially in Clinical and Experimental Obstetrics and Gynecology, which in contrast to many other peer reviewed journals, allows a greater amount of space for more in depth description of case reports.
To what extent should the gynecologist become involved with headaches? It certainly seems appropriate for the gynecologist to refer the women to a neurologist to exclude more serious etiologies, e.g., brain tumors or aneurysms, etc. If the neurologist does not find a serious etiology and prescribes therapy that is effective and does not cause side effects, the gynecologist does not need to intervene. If the gynecologist prefers, one could try to make the treating neurologists or internists aware of this disorder of sympathetic nervous system hypofunction and defer to the neurologist, if that specialist is willing to provide treatment with dextroamphetamine sulfate. If not, the gynecologist, who has assumed the role as the primary care physician for women, in our opinion, should then treat the patient themselves.

Other than brain tumors or other vascular space occupying lesions, are there any other types of conditions causing headaches that should not be treated with dextroamphetamine sulfate? A 34-year-old woman presented with severe headaches and papilledema [12]. Fortunately she was not found to have a tumor but instead was diagnosed with intracranial hypertension (pseudotumor cerebri) [12]. She had only mild relief from the combination of topiramate and acetazolamide. She came to our practice having moved from another state to take over her management for hypothyroidism. She complained in addition to the headaches of chronic fatigue, backache, and inability to lose weight. She was advised that hypofunction of the sympathetic nervous system could be the cause of the backache and the chronic fatigue and inability to lose weight despite dieting [9, 13, 14]. Dextroamphetamine sulfate therapy completely alleviated her headaches and chronic fatigue and backache within one month of therapy [12]. Several months later she returned for her appointment with the neuro-ophthalmologist and advised her of the tremendous improvement with this new therapy. The specialist did not ask her any questions about this new therapy but merely did a fundoscopic examination, advised her that the papilledema was completely gone, and to return in six months for another fundoscopic examination. Thus, if the specialists are not interested in this new treatment, it behooves the woman’s gynecologist, who should be her gatekeeper for medical problems, to institute therapy.

The case of intracranial hypertension illustrates another key point and that is that the gynecologist should be aware of all of the different clinical manifestations that are associated with the sympathetic neural hyperalgesia edema syndrome. The sympathetic nervous system controls the temperature regulation system. Dextroamphetamine sulfate has been found to be effective in treating vasomotor symptoms even in the presence of normal estrogen in women with estrogen deficiency who are either reluctant to take estrogen or where it is contraindicated [15, 16]. Because of weight issues and fear of estrogen causing weight gain, a 44-year-old woman sought alternative therapy [17]. She also complained of severe headaches that would occur two weeks of the month, but each time she flew on an airplane (which were so severe she could not fly). Both her vasomotor symptoms and headaches completely disappeared following dextroamphetamine sulfate therapy and now she has no problem on airplanes [17].

Thus, to date, there does not seem to be any type of headaches, other than tumors or aneurysms that will not respond to dextroamphetamine sulfate. Even headaches related to TMJ syndrome seem amenable, as evidenced by the present case report. Of course one does not know for sure if the TMJ dislocation was responsible for the headaches or if she has a headache syndrome strictly from hypofunction of the sympathetic nervous system, and the TMJ by itself would not have caused headaches. Nevertheless, institution of dextroamphetamine sulfate saved this woman an expensive surgical procedure that involved breaking her jaw. The surgery that was offered was explained by the surgeon that there was only a small chance it would likely help her headaches.

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Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)
e-mail: laurie@ccivf.com
Failure of laparoscopic Vecchietti procedure in a woman with androgen insensitivity syndrome

D. Sawan, N. Sahly, H. Abduljabbar, A.A. Rouzi

Department of Obstetrics and Gynecology, King Abdulaziz University, Jeddah (Kingdom of Saudi Arabia)

Summary

The authors describe a case with androgen insensitivity syndrome (AIS) who underwent the laparoscopic Vecchietti procedure for creation of a neovagina. Postoperatively, the patient achieved anatomic success, with a vaginal length of about eight cm, and she was advised to use vaginal dilators after discharge. The patient reported improved sexual function, but presented about six months later for shortening of her vagina and difficult vaginal intercourse. Physical examination revealed an obliterated vaginal canal about two cm long. Further examination revealed lack of vaginal epithelization. The patient was instructed to continue using vaginal dilators in combination with estrogen cream; however, the patient did not achieve a vaginal length > two cm. The authors believe that the laparoscopic Vecchietti procedure may not be appropriate for women with AIS due to lack of epithelization.

Key words: Androgen insensitivity syndrome; Vecchietti procedure; Epithelization.

Introduction

Surgical vaginal creation may be necessary in cases where the vagina is short or absent. Such is the case in patients with Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome and complete or partial androgen insensitivity syndrome (AIS). While the use of non-surgical methods such as dilators have been shown to be effective in patients with a short or absent vagina, [1] these may not be suitable for some women. Women who have had several vaginal surgeries may not benefit from dilation owing to the incapacity for the vaginal tissue to conveniently stretch by dilators alone. Moreover, the use of vaginal dilators carries deep psychological and emotional implications that make it intrusive, with many women reporting that dilators remind them of their abnormality [2]. For these women, surgical reconstruction of a neovagina may be beneficial. Current methods for vaginal reconstruction have replaced the traditional surgical procedures where the neovaginal space was lined with a split-thickness skin graft (McIndoe-Reed procedure) [3] or with a section of the intestine [4]. Among the recent methods, the Vecchietti and Davydov techniques have been used and both procedures are now performed laparoscopically. Previous reports have shown low complication rates associated with these procedures [5, 6]. In addition, some studies have reported the Vecchietti procedure to be successful in patients with AIS [7, 8]. The authors present the case of a patient with AIS who had vaginal agenesis in whom the laparoscopic Vecchietti procedure failed.

Case Report

The patient is an 18-year-old, divorced woman who was referred to King Abdulaziz University Hospital for management of AIS. At the age of 14 years, she complained of amenorrhea, but she was reassured. The patient presented later at the age of 18 years with complaints of painful and difficult intercourse. Physical examination revealed the absence of the uterus. A diagnosis of AIS was confirmed and laparoscopic creation of a neovagina using Vecchietti’s method was offered. She underwent uneventful laparoscopic Vecchietti procedure as described before [6]. Post-surgery, the patient was noted to have a vaginal length of about eight cm. She was instructed to use vaginal dilators after discharge. Initially, she reported improved sexual function but presented about six months later with a complaint of shortening of her vagina and difficult vaginal intercourse. Upon examination, the vaginal canal was obliterated and measured about two cm. Further examination revealed lack of vaginal epithelization. The patient was instructed to continue using vaginal dilators in combination with estrogen cream; however, during subsequent visits, the patient did not achieve a vaginal length more than two cm.

Discussion

AIS is characterized by evidence of feminization of the external genital organs at birth, abnormal secondary sexual development in puberty, and infertility in individuals with a 46,XY karyotype [9]. Patients with AIS demonstrate a range of defects in androgen action. Three distinct forms
of AIS have been identified based on phenotype [9]: 1) complete androgen insensitivity syndrome (CAIS) where the patient has typical female external genital organs; 2) partial androgen insensitivity syndrome (PAIS) where the patient has predominantly female, predominantly male, or ambiguous external genital organs; 3) mild androgen insensitivity syndrome (MAIS) where the patient has typical male external genitalia.

The characteristics of the present patient were consistent with CAIS. MRKH syndrome can also mimic CAIS. Similar to patients with CAIS, those with MRKH demonstrate primary amenorrhea, underdeveloped vagina, and normal breast development; however, the 46, XX karyotype is critical in differentiating both syndromes, [10] which was the case in the present patient.

While vaginal dilation is reportedly the treatment of choice in patients with short vaginal length, surgery is recommended when dilation fails. However, the timing and nature of the intervention is critical and surgeons need to work closely with the patient and other physicians involved in the care of the patient [9]. In the present case, vaginal dilatation was not explored initially, as the patient was married and had already engaged in sexual activity. In addition, the authors believed that delaying surgery may have had a negative impact on the patient’s social and mental well-being and, consequently, jeopardized the functional success of the operation. Besides, the patient must be motivated and be aware of the procedure as well as of the need for a postoperative phase.

The laparoscopic approach for creating a neovagina with the Vecchietti method has been reported to be simple, safe, and effective [5, 6]. Anatomic success, with vaginal lengths > six cm and good functional outcomes have been described in one case series of 86 patients with vaginal agenesis [11]. In their report, the authors found that 84 of the patients achieved a vaginal length > six cm within eight days after laparoscopic Vecchietti; after six months, the length of the neo-vagina was 6.5 cm in four patients and seven cm in all remaining patients. In addition, 82 of the patients in their series showed evidence of vaginal epithelization within six months after surgery. While in the present case, the patient initially achieved a vaginal length of eight cm and reported improved sexual function, the vaginal canal was obliterated and measured about two cm six months after the procedure. Further examination revealed lack of vaginal epithelization, and continued use of vaginal dilators in combination with estrogen cream did not result in anatomic success.

Finally, while there are isolated reports [7, 8] of successful vaginoplasty with the Vecchietti laparoscopic procedure in patients with AIS, the present case prompts the authors to believe that further clinical evaluation is required to explore this treatment option in AIS. In addition, the decision for surgery should be individualized to the patient and the patient’s desire for vaginoplasty.

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Address reprint requests to:
A.A. ROUZI, M.D.
P.O. Box 80215
Jeddah 21589 (Kingdom of Saudi Arabia)
e-mail: aarouzi@gmail.com
Intra-amniotic methotrexate in cervical pregnancy treatment

Clinic of Gynecology and Obstetrics, Clinical Center Niš, Niš (Serbia)

Summary

Cervical pregnancy is an extremely rare condition with potential devastating consequences if not diagnosed and treated early enough. The authors present a case with ultrasound images of early cervical ectopic pregnancy in a woman with a history of previous cesarean section who was successfully treated with intra-amniotic methotrexate (MTX).

Key words: Cervical pregnancy; Methotrexate.

Introduction

Cervical pregnancy is extremely rare form of ectopic pregnancy, which is often associated with significant morbidity and endangering influence on future fertility. It accounts for less than one percent of ectopic pregnancies. The incidence is approximately one in 9,000 deliveries [1]. Its etiology is still unclear; chromosomal abnormalities as well as local pathology related to previous cervical or uterine surgery may play a role given an apparent association with a prior history of curettage or cesarean delivery [2]. Also there are reports of association in pregnancies achieved through assisted reproductive technologies; it occurs in 0.1 percent of in vitro fertilization pregnancies [3]. The diagnosis of cervical pregnancy is commonly delayed and is often made intraoperatively in the presence of massive blood loss, necessitating an emergency hysterectomy in ~50% of cases. Early diagnosis has been improved by ultrasonography, with a consequent decrease in morbidity and mortality [4]. In an attempt to avoid hysterectomy and preserve fertility, a more conservative therapeutic approach was developed, including chemotherapy, cerclage, hypogastric iliac artery ligation, and arterial embolization under angiographic control. [5].

In this report, the authors present a conservative approach, using transabdominal intra-amniotic instilling of methotrexate (MTX) for cervical pregnancy treatment, after unsuccessful intramuscular use.

Case Report

A 33-year-old woman, gravida 3, para 1, was admitted to the present department at six weeks gestation with painless vaginal bleeding. In previous birth, section cesarean was performed, and two years prior she had a miscarriage. Vital signs were stable and the abdomen was soft and not tender. Pelvic examination revealed a barrel-shaped uterine cervix with minimal bright bleeding protruding through a closed external os (Figure 1). The uterus was slightly enlarged and had no adnexal masses. Transabdominal and transvaginal ultrasound examinations confirmed the presence of a cervical pregnancy with fetal pole and fetal cardiac activity (Figure 2). Quantitative beta-human chorionic gonadotrophin (hCG) concentration was 8,620 mIU/mL on admission, and almost twice that (15,800 mIU/mL) two days later. In an attempt to preserve fertility, the patient was offered the patient conservative management with i.m. MTX. The potential risks and alternative methods of treatment were explained to her, and written informed consent was obtained. The most commonly used treatment regimen in the present department was applied. This consisted of i.m. MTX one mg/kg and folic acid five mg given orally every other day for four days. After completion of treatment, however, the ultrasound examination revealed an unchanged gestational sac size with persistent fetal cardiac activity. On the basis of these findings and the patient’s haemodynamically stable state, the authors decided to try direct intra-amniotic MTX administration. Under transabdominal ultrasound control, a 22 ga/12 cm needle was placed within the amniotic cavity, ten ml of amniotic fluid were withdrawn, and 40 mg MTX was instilled. After four days the procedure was repeated. During the next few days the beta-hCG level decreased slightly measuring 10,230 mIU/mL at one week, with the concomitant disappearance of fetal cardiac activity. One week later, a collapsed gestational sac was demonstrated (Figure 3).

Six months later hysteroscopy was performed and showed no signs of cervical damage (Figure 4).

Discussion

In the present patient, the fetus was implanted below the previous cesarean section scar. The uterus was empty and the gestational sac showed evidence of fetal heart rate at six to seven weeks in two different ultrasounds. Color Doppler also confirmed blood flow around the gestational sac. Unlike true cervical pregnancy, cervical abortion is suggested by the body of the uterus being larger than in the nongravid state owing to the recent loss of the intrauterine sac. Two ultrasounds in the present patient showed evidence of fetal
heart activity and placement of the gestational sac in the cervix below the scar of the previous cesarean section [6]. Patients with cervical pregnancy classically present with painless first trimester vaginal bleeding. Cervix was closed, enlarged, and tender. Estimated gestational age based on last menstrual period was six weeks and two days. A few days should distinguish the cervical abortion by the transience of the sac if the diagnosis is in doubt [7]. Treatment choices may be divided into five categories: tamponade, reduction of blood supply, excision of trophoblastic tissue, intra-amniotic feticide, and systemic chemotherapy [8]. In most reported cases of cervical pregnancy, treatments from more than one category are used [9]. Treatment with MTX chemotherapy in patients with either viable or nonviable cervical pregnancies at < 12 weeks’ gestation carries a high success rate for preservation of the uterus. The present authors suggested antimetabolite treatment (MTX), although studies have shown unsatisfactory results if serum beta hCG is more than 10,000 IU/L, which was the case in the present patient [10]. Cervical pregnancy is a rare condition that can be life-threatening if not diagnosed and treated early during the course of pregnancy. Increasing trend of cesarean sections and use of other invasive methods such as intrauterine device and in vitro fertilization seem to currently contribute to a higher prevalence of cervical pregnancies [11]. Cervical pregnancies require early diagnosis and management which are necessary in preserving patient’s fertility without significant complications.

References

Intra-amniotic methotrexate in cervical pregnancy treatment


Address reprint requests to:
M. STEFANOVIĆ, M.D.
Nikole Kopernika 21
18000 Niš (Serbia)
e-mail: mila9@open.telekom.rs
Laparoscopic temporary clipping of uterine and ovarian arteries for the treatment of interstitial ectopic pregnancy

C. Kart1, S. Guven1, E.S. Guvendag Guven1

1 Department of Obstetric and Gynecology, Karadeniz Technical University, School of Medicine, Trabzon (Turkey)

Summary

Purpose of investigation: To assess the effect of laparoscopic temporary clipping of uterine and ovarian arteries for the treatment of interstitial ectopic pregnancy. Materials and Methods: A 29-year-old woman with vaginal bleeding and pelvic pain was admitted to the current clinic. She had secondary amenorrhea for nine weeks. Transvaginal ultrasonography revealed normal empty uterus and right interstitial ectopic pregnancy with viable embryo. Laparoscopic temporary clipping of uterine and ovarian arteries, interstitial pregnancy resection, and primary myometrial suturing was performed. Results: Following dissection Latzko pararectal space for the visualization of both uterine arteries, four vascular clips were placed (two to uterine arteries, two to infundibulopelvic ligaments). Excision of interstitial pregnancy and primary myometrial suturing was performed with minimal blood loss. The patient was discharged from the hospital after one day without any remarkable complications. Conclusions: To the best of the authors’ knowledge, this is the first case of interstitial pregnancy that was successfully treated by temporary laparoscopic clipping of uterine and ovarian vessels prior to interstitial ectopic pregnancy resection.

Key words: Interstitial ectopic pregnancy; Laparoscopy; Clipping; Uterine artery; Ovarian artery.

Introduction

Interstitial pregnancy is an ectopic pregnancy that is implanted in the interstitial part of the fallopian tube, which is defined as proximal tubal segment within the muscular wall of the uterus. Interstitial pregnancy is a rare condition accounting for only 2-4% of tubal pregnancies [1]. However, because of diagnostic pitfalls and relatively late diagnosis, the maternal mortality rate was reported as 2.5% [2, 3]. Rupture of such rare pregnancies may cause dangerous bleeding and maternal mortality. Early diagnosis and interventions may give chance to treat such dangerous situation. Conventional treatments for interstitial pregnancy have ranged from cornuostomy to hysterectomy by laparotomy and rarely by laparoscopy. Surgical treatment also requires experienced endoscopist or surgeon, because of the risk of intractable hemorrhage [1].

To the best of the authors’ knowledge, this is the first such report that a case of interstitial pregnancy was treated by temporary laparoscopic clipping of uterine arteries and infundibulopelvic vessels prior to gestational sac and the placental tissue removal, and the interstitial part of fallopian tube repaired.

Case Report

A 29-year-old, gravidity 3, parity 1, missed abortion 1 woman with pelvic pain and vaginal bleeding was admitted to current university hospital. Her obstetric history was unremarkable, except one history of cesarean delivery. She had secondary amenorrhea for nine weeks and serum level of hCG was measured as 43,367 mIU/ml. Initial transvaginal sonography revealed normal empty uterine cavity and a gestational sac 30×35×30 mm in diameter having viable fetal pole (CRL 21.2 mm /eight weeks and two days) and yolk sac, located just adjacent to the fallopian tube interstitial region (Figure 1).

Patients’s preoperative hemoglobin concentration was 12.8 g/dL (range 12−16), hematocrit was 33.8% (range 30−50), and platelet count was 257,000/µL (range 140,000−440,000). Following preoperative evaluation and suggestive diagnosis of interstitial ectopic pregnancy, the patient underwent laparoscopy. Gynecologic laparoscopy revealed an enlarged and prominent right interstitial region almost eight cm in diameter (Figure 2). Both ovaries and left tube were apparently normal.

The posterior broad ligament was opened via three-cm peritoneal incision and the ureter with surrounding periton was isolated and positioned away form the uterine artery. To minimize blood loss, Latzko pararectal spaces were entered to visualize uterine arteries. Following identification of internal iliac artery and ureter, a plastic atraumatic bulldog clip (Figures 3a-b) was applied across the associated uterine artery. Then, the same procedure was repeated on the contralateral side. Two additional plastic atraumatic bulldog clips were also applied to the infundibulopelvic ligaments lateral to the ovary on each side without any dissection (Figure 4). Following vascular clips application, an almost four-cm linear incision was made to uterine wall and gestational sac and placenta were removed. The minimal bleeding from the bed of interstitial pregnancy was controlled with the aid of bipolar forceps. The uterine defect was repaired with primary intracorporeal myometrial suturing using 2.0 polyglactin 910 suture (Figure 5). Finally all four plastic atraumatic bulldog clips were removed (Figures 6, 7). Total
measured operative blood loss was almost 50 ml. Postoperative patient’s hemoglobin concentration was 12.2 g/dL (range 12–16), hematocrit was 33.5% (range 30–50), and platelet count was 244,000/µL (range 140,000–440,000).

The patient was discharged from the hospital after one day. Four weeks after discharge the woman was in good health status and the serum hCG level was measured and found below five mIU/ml.

Discussion

Here, the authors report a case of interstitial pregnancy which was successfully treated via laparoscopic temporary application clips to uterine and ovarian arteries, removal of gestational sac and placenta, and primary myometrial suturing.

Temporary uterine and ovarian vascular clipping is a novel endoscopic treatment strategy that gives a change to reduce the intraoperative blood loss to minimal amount and to decrease the chance of hysterectomy because of intractable bleeding.

Interstitial ectopic pregnancy is a rare and sometimes misdiagnosed form of ectopic pregnancy. The diagnosis and also the surgical treatment of this condition are challenging. Clinicians almost always are afraid of intractable intra-abdominal bleeding because of sudden uterine rup-
ture and intraoperative uncontrollable bleeding. Because of this known complication, treatment of this rare condition requires experienced gynecologist and clinicians usually prefer open laparotomy techniques instead of laparoscopy. However, laparoscopy has the advantages of minimal invasive surgery such as early ambulation, early discharge from the hospital, and decreased postoperative pain.

The possible operative intractable bleeding risk of this form of ectopic pregnancy forces endoscopist to search for new endoscopy techniques. In the literature there have been some reported techniques to overcome such complication. Vasopressin injection into myometrium was proposed to limit the intraoperative blood loss. However, vasopressin associated vasoconstriction is limited by short half-life; hypertension and bradycardia are the possible maternal adverse affects that limit to use this strategy [4]. The second treatment strategy was occlusion of ascending branch of uterine artery. This technique may not fully control bleeding, may decrease the risk of subsequent, and include postoperative pain because of tissue ischemia [1, 5]. Suture placement below myometrial resection, purse-string suture technique [6], and suture loop placement [7] were used to decrease intraoperative blood loss, but these techniques may cause disruption of anatomy and possible tubal occlusion which may decrease the future risk spontaneous pregnancy [1].

Electrocoagulation may also be used to control the hemorrhage on the bed of interstitial ectopic pregnancy site [8]. However, electrocoagulation may damage the myometrium and increase risk of subsequent rupture in future pregnancies [1].

Interstitial ectopic pregnancy is a disease of reproductive aged woman. These women want to preserve their future fertility for planning future pregnancies. Based on this patient’s desire, a new technique “laparoscopic permanent occlusion of unilateral ascending branch of uterine artery and the communication of the ovarian artery” was described by Cheng et al. They suggested that this technique may minimize blood loss and allow treating interstitial ectopic pregnancy conservatively [9]. The present authors believe that the permanent closure of uterine artery may interfere with the future fertility potential by decreasing the blood supply of uterus. Uterine artery embolisation (UAE) studies reported that women following UAE have increased risk of infertility, miscarriage, preterm delivery, placental problems, and malpresentation [10, 11]. Furthermore occlusion of communication of ovarian artery may also decrease the blood supply of ovary, decrease the ovarian reserve, and decrease the chance of future pregnancy. The permanent occlusion of uterine artery may also cause postoperative ischemia associated pelvic pain.

The present authors’ new technique gives chance to the clinicians to control intraoperative intractable bleeding and to treat interstitial ectopic pregnancy. The temporary bilateral uterine and ovarian artery clippings decreased the intraoperative bleeding to minimal amount and allowed the removal of interstitial ectopic pregnancy with the full chance of conserving interstitium and fallopian tube for future spontaneous pregnancy. Furthermore, preoperative and postoperative hemoglobin/hematocrit results also showed the reduced blood loss during the operation. Removing the vascular clips at the end of surgery increased patient postoperative comfort such as experiencing postoperative tissue ischemia associated to pelvic pain. This technique was successfully applied to myomectomy procedures and reported valuable results [12].

To the best of the authors’ knowledge this is the first report using laparoscopic temporary uterine and ovarian artery clipping for the treatment of interstitial ectopic pregnancy. This case report suggests clinicians to use this technique for the endoscopic treatment of this rare clinical entity to overcome interstitial ectopic pregnancy associated complications.

References


Address reprint requests to:
S. GUVEN, M.D.
KTÜ Tip Fakültesi, Farabi Hastanesi
Kadın Hastalıkları ve Doğum ABD
61080 Trabzon (Turkey)
e-mail: drsuleymanguven@yahoo.com
Incarceration of gravid uterus by growing subserosal myoma: case report

S.C. Kim, Y.J. Lee, J.E. Jeong, J.K. Joo, K.S. Lee

Department of Obstetrics and Gynecology, medical research institute Pusan National University School of Medicine, Busan (Korea)

Summary

Incarceration of gravid uterus is a rare condition, occurring in one in 3,000 to 10,000 pregnancies during second trimester. Incarceration of uterus can cause several complications, such as uterine rupture, labor dystocia, and uncontrollable postpartum hemorrhage. Early diagnosis is important to prevent these complications, but there are no standard treatments of incarceration of gravid uterus. The authors present a case report of incarceration of gravid uterus caused by growing subserosal myoma, which was treated with myomectomy during second trimester.

Key words: Retroverted gravid uterus; Subserosal myoma; Uterine incarceration.

Introduction

Approximately 11% of pregnant women have a retroverted uterus [1]. Usually it changes to spontaneously antverted upward position by 14 weeks of gestation [2]. When retroversion persists beyond the second trimester, the uterine corpus becomes entrapped in the hollow of the sacrum. This complication is called uterine incarceration [3]. The incidence of uterine incarceration during second trimester has been reported in one in 3,000 to 10,000 pregnancies [4, 5]. Predisposing factors of uterine incarceration are pelvic adhesions, endometriosis, uterine malformation, leiomyoma, and pelvic tumors [6]. Therefore if the patient has these factors, the obstetrician has to consider possibility of uterine incarceration. If the obstetrician misses to diagnose this condition, it can lead to some emergencies, such as uterine rupture, labor dystocia, and uncontrollable postpartum hemorrhage [7].

Here the authors present a case of an incarcerated gravid uterus caused by growing subserosal myoma and myomectomy was performed for correction of uterine incarceration at second trimester, and the baby was successfully delivered vaginally at 39 weeks of gestation.

Case Report

A 29-year-old married woman, gravid 0, had visited the outpatient clinic for gynecologic check-up. By ultrasound, she was diagnosed with a subserosal myoma which was located at anterior part of uterus, seven cm sized (Figure 1), but she did not have any specific symptoms, and the present authors recommended regular follow up. After three months, she returned for antenatal care with intrauterine pregnancy 6+4 weeks of gestation. By ultrasonographic scanning, the authors found the fetus with normal fetal heart beats and the crown-rump length was six weeks of gestation size (0.67 cm) and the uterus was in an antverted state. However the subserosal myoma had grown to 10.85 cm. The antenatal routine check-up results were all within normal range.

For quad test, she returned to the outpatient clinic at 15+5 weeks of gestation. The patient complained of constipation and low abdominal discomfort. Ultrasonographic scan revealed that the subserosal myoma was increased to 13.45 cm and the uterine position was changed to retroverted. The authors checked magnetic resonance imaging (MRI) of the pelvis to identify exact position of uterus. It revealed a uterus that was retroverted by a large subserosal myoma with dimensions of 20 x 15 cm (Figure 2). The cervix was extended anteriorly and the body of uterus was seen with an acute retroverted angle. So the authors diagnosed incarcerated retroverted gravid uterus caused by huge subserosal myoma.

They expected natural repositioning of the uterus following increasing uterine size with advancing gestational age at outpatient clinic. However, the symptoms of incarcerated uterus, lower abdominal pain, and constipation worsened, therefore the patient was admitted for manual reduction, but the traction of uterine cervix by grasping forceps failed and then the authors performed myomectomy with general anesthesia. In supine position, low midline incision about ten cm was done. After opening of peritoneum, the authors found a subserosal myoma on the anterior surface of gravid uterus and the myoma was expelled from abdominal cavity manually (Figure 3). The stalk of subserosal myoma was ligated with vicryl 1-0 suture and the subserosal myoma was removed. After removing the subserosal myoma, the uterus was naturally repositioned to antverted state. Postoperative ultrasound scan showed normal fetal heart rate and no intrauterine or subplacental hematoma. After surgery, the patient’s symptoms improved without complications. The pregnancy progressed uneventfully and normal spontaneous vaginal delivery was done at 39 weeks of gestation. The patient gave birth to a...
healthy female baby weighing 3,430 g and Apgar score were 7 and 9 at one and five minutes respectively. The patient and baby were discharged postpartum second day without complication.

Discussion

The incarceration of retroverted gravid uterus is when the uterine fundus remains in the lower pelvis below sacral promontory and both bladder and cervix are elongated and pulled in to abdominal cavity. Approximately 11–19% of pregnant women have a retroverted uterus [2]. Usually retroverted uterus changes to anteverted state by 14 weeks of gestation [2]. However the incidence of uterine incarceration during second trimester has been reported in one in 3,000 to 10,000 pregnancies [4, 5]. In the present case, the uterine position was anteverted before pregnant and early pregnant period, but the uterine position was changed to retroverted due to rapid growth of subserosal myoma. The present authors did not find reports of incarceration by rapid growing myoma in their PubMed research. Because of anatomical changes of bladder and cervix, the most common symptoms of uterine incarceration are abdominal pain and voiding difficulty. The other symptoms are rectal pressure, tenesmus, constipation, and vaginal bleeding [3]. The complications of persistent incarcerated uterus are fetal loss, preterm labor, uterine rupture, and postpartum hemorrhage [7]. In this case, lower abdominal pain and constipation were main symptoms and worsened by progress of pregnancy. For these reason, early diagnosis of incarcerated uterus is important. The risk factors of incarcerated uterus are pelvic adhesion related to previous surgery, pelvic inflammatory disease, endometriosis, large pelvic mass, and uterine malformation [6].

Uterine myoma is the most common benign uterine mass. The incidence of uterine myoma during pregnancy is 0.1-3.9% [8]. Mostly, uterine myoma do not cause problems during pregnancy. The size of uterine myoma increases during first trimester and at second and third trimester, the myoma size was stabilized [8]. Conservative management is the principle of uncomplicated uterine myoma during gestation period. Therefore, surgical treatment is not a standard method for women who want to persist in their pregnancy.

For the successful management, the correction of uterine incarceration has to be performed before 20 weeks of gestation to prevent development of complications. There are several methods for repositioning of incarcerated uterus, but non-invasive method should be considered first [4].

Treatment techniques are applied to patients according to the gestational age [7]. In the late first or early second trimester, spontaneous repositioning of incarcerated uterus is possible [9]. One of general methods used in first trimester is knee-chest position intermittently over several
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hours with an empty bladder [10]. If spontaneous reduction is not successful, manual repositioning may then be attempted. Manual manipulation through the posterior fornix should be considered, while the patient is in the dorsal lithotomy position. If this method is unsuccessful, manual manipulation can attempted once more under an anesthetic condition. It has been found that epidural anesthesia is a reasonable approach to the initial anesthetic management for reduction of an incarcerated uterus [10]. Another manual repositioning method is attempted by pulling down the cervix and maintaining rectal pressure on the fundus at the same time. In the present case, the cause of uterine incarceration was due to the mass effect of the subserosal myoma, hence the possibility of manual reduction was thought to be low. The present authors attempted manual traction of uterine cervix only one time.

In the present case, uterus was incarcerated because of rapid growth of the subserosal myoma. The present authors therefore recommend that myoma should be observed more carefully during pregnancy, especially in a rapid growing case. The incarceration of gravid uterus by rapid growing subserosal myoma might be corrected only by myomectomy and the patient had no problem during postoperative and pregnant period and the baby was born successfully by vaginal delivery. The present authors have reported this case with a short review of the articles.

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Address reprint requests to:
J.K. JOO, M.D.
Department of Obstetrics and Gynecology
School of Medicine Pusan National University Hospital
179 Gudeok-Ro, Seo-Gu
602-739 Busan (Korea)
e-mail: jongkilj@hanmail.net
Laparoscopic subtotal hysterectomy due to giant uterine fibroids: a case report

J.Y. Ruan, H.Q. Chen, Y.H. Gong, G. Shi, H. Wang

Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu (China)

Summary
The laparoscopic subtotal hysterectomy (LSH) was given to a patient whose uterus was about seven-month pregnancy because of fibroids. The biggest problem was the operation space and visual field was too narrow. Different from the usual procedure we do, we morcellated the uterus at the beginning to expand the space. Loop ligation of the uterine isthmus was adopted to block uterine arteries before morcellating the uterus. After the adnexa exposed totally, we started to cut off the round ligaments, proper ligaments and fallopian tubes like usual. It was the first time we did LSH for so giant uterus in our hospital, although which was usually suitable for the uterus smaller than four-month pregnancy. But if the uterine arteries can be blocked effectively at the beginning, the uterus can be morcellated and the space will be enlarged. The laparoscopic subtotal hysterectomy will also be completed successfully.

Key words: Laparoscopic subtotal hysterectomy, uterine fibroids, loop ligation.

Introduction
Semm completed Laparoscopic Subtotal Hysterectomy (LSH) successfully in 1991[1]. With the improvement of surgical instruments, the advantages of LSH have increased quickly. LSH can reduce the trauma and bleedings, shorten the length of hospital stay, keep the integrity of vagina and reduce the risk of pelvic floor relaxation [2]. But LSH also has shortcoming that is the LSH is limited by the uterine size [2]. It is usually suitable for the uterus which size is smaller than four-month pregnancy. But in this case we completed LSH on uterus sized seven-month pregnancy.

Case Report
A 53-year-old woman was admitted to the hospital on May 18, 2011 because of giant uterine fibroids. The cycle of the menstruation was still normal but the quantity had increased 4 years ago. But she didn’t have any abdominal pain. Physical examination showed abdominal obesity with Body Mass Index of 29.5 kg/m². Scars covered 30% abdominal wall and umbilicus because of the deep two degree burn when she was 4-year-old (Figure 1). Pelvic examination showed normal and smooth cervix. The size of uterus was about seven-month pregnancy. Ovaries were impalpable due the obese and scared abdominal wall. Ultrasound examination revealed that the uterus enlarged to 11.9 cm×18.8 cm×18.8 cm. The giant fibroids located in the anterior uterine wall with size of 9.1 cm×16.1 cm×16.8 cm. Both sides of the ovaries could be detected. The cervical cytological examination was normal. The preoperative diagnosis was giant uterine fibroids.

The Laparoscopic subtotal (supracervical) hysterectomy (LSH) was performed on May 19, 2011. We placed one optical trocar and three trocars for operating in the lower quadrant abdomen. The optical trocar (Φ10mm) was 3 cm below the xiphoid on the midline. The No.1 operating trocar (Φ10mm) was 10 cm above the left anterior superior iliac spine on the anterior axillary line. The No.2 trocar (Φ5mm) was 2 cm inside from the right anterior superior iliac spine. The No.3 trocar (Φ5mm) was 2 cm inside from the left anterior superior iliac spine.

One end of 1-0 absorbable suture was put into abdominal cavity from the No.2 trocar. The other end was fixed outside of the abdomen. Pull up the uterine corpora to expose the uterine isthmus. The end of the suture which was put into the abdominal cavity was pulled out from the No.2 trocar after surrounding the isthmus one circle. Knot two ends to form a loop. Use the knot pusher to push the knot to the isthmus and tighten up the loop continuously. Change the No.1 trocar into Φ18mm trocar. Put the morcellator into No.1 trocar to morcellate the uterus from the bottom (Fig 2). When the adnexa exposed, we began to cut off the both sides of the round ligaments, proper ligaments and fallopian tubes from the No.3 trocar. Pull up the uterus to expose the uterovesical peritoneal reflection.Open the uterovesical peritoneal reflection.Push down the bladder 1 cm. Use the second loop to ligate the isthmus inside of the both adnexa and cut off the first loop to release the ovaries’ blood vessels. Then keep on morcellating the uterus till the the stump was about 2 cm. Clip the stump to 1 cm and cauterize the uterine blood vessels. At last make the third loop and ligate the stump (Figure 3). The Laparoscopic subtotal hysterectomy was finished successfully.

The intraoperative bleeding was estimated as 600 ml. The procedure lasted three hours and 50 minutes. It took about two hours to morcellate the uterus and fibroids. The total weight of the removed uterus and fibroids was 2500 grams (Figure 4). The patient recovered quite well and was discharged 3 days later. Pathological result was uterine leiomyoma with degeneration. We did not observe any complication and potential disseminated leiomyomatosis during 3-year follow-up.
Discussion

The LSH is usually applied for the uterus with enlarged size up to four-month pregnancy. Otherwise the visual field and operation space will be too limited. However, we managed the LSH on the uterus sized seven-month pregnancy. We suggested laparoscopy because there were a lot of scars on the abdominal wall, which was vulnerable to wound infection after laparotomy. Secondly, given no laparotomy history, the adhesion in the abdominal cavity might not be serious.

We chose laparoscopic subtotal hysterectomy but not laparoscopic total hysterectomy because the cervix was normal and laparoscopic subtotal hysterectomy required less operative dissection of the bladder, ureter, bowel, and uterine artery [3].

The biggest problem of this operation was the narrow operation space and visual field caused by the giant uterus. We must lessen the uterus in order to expand the space. The usual procedure of LSH is cutting off round ligaments, proper ligaments, fallopian tubes, opening the uterovesical peritoneal reflection, cutting off the uterine arteries and morcellating the uterus at last. But this time we needed morcellate the uterus firstly. Morcellating the uterus before the uterine arteries cut off will cause serous bleeding. So we decided the loop ligature of the uterine isthmus to block the uterine arteries, thus we morcellated the uterus and fibroids without worrying about the bleeding.

Loop ligature is safe, effective and can reduce operation time [4]. It doesn’t need freeing the uterine arteries, avoiding vascular injury. It also avoids thermal damage [5]. After the loop ligature, the pelvic cavity and the vagina is not interlinked. So we don’t need to close the peritoneum which makes the operation more simply [6]. It is very important
to tighten up the loop continuously when morcellating which can reduce the bleeding during operation. Double loop ligatures can reduce the risk of postoperative bleeding we think.

Conclusion

It was the first time we did the LSH for so giant uterus, however it might be considered impossibly to complete. But if the operation space and visual field could be enlarged effectively, giant uterus like this case can also be removed successfully. The practiced hands and good cooperation are both important for the operation which can shorten operation time and reduce injuries.

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Address reprint requests to:
H. WANG, M.D.
Department of Obstetrics and Gynecology
West China Second University Hospital
Sichuan University
Renmin South Road, Chengdu (China)
e-mail: jiajiasheng@163.com
Holt Oram syndrome: a case report and review of the literature

G. Virdis¹, M. Dessole¹, S. Dessole¹, G. Ambrosini², E. Cosmi², P.L. Cherchi¹, G. Capobianco¹

¹ Gynecologic and Obstetric Clinic, Department of Surgical, Microsurgical and Medical Sciences. University of Sassari, Sassari
² Gynecologic and Obstetric Clinic, University of Padua, Padua (Italy)

Summary
Holt Oram syndrome is a rare autosomal dominant syndrome on average, of varying severity, which may result in heterogeneous pictures, predominantly with involvement of the bony segments of the upper limbs and the cardiovascular system. The syndrome is caused by mutations in two genes of the T-box (TBX5, 601 620 and TBX 3) located on the 12q24.1p. The authors report a case and review the literature.

Key words: Holt Oram syndrome; Obstetric ultrasound.

Introduction
Holt Oram syndrome is a rare autosomal dominant syndrome on average, of varying severity, which may result in heterogeneous pictures, predominantly with involvement of the bony segments of the upper limbs and the cardiovascular system. Mary Holt and Samuel Oram described it for the first time in 1960, and since then several authors [1-13] have reported more than 300 cases. The presence of cardiac abnormalities, muscle, and bone inspired a number of names for this syndrome, such as “heart-hand syndrome”, “syndrome heart-upper”, and “upper limb cardiovascular syndrome”. The syndrome is caused by mutations in two genes of the T-box (TBX5, 601 620 and TBX 3) located on the 12q24.1p. The TBX5 gene is only involved in the development of the upper limb. The prevalence is estimated to be 0.95 cases per 100,000 births, shows a penetrance of 100%, with a recurrence risk of 50%. About 85% are due to new mutations [1-12, 14-19].

Case Report
The authors report here a case of a patient with a syndromic fetus admitted to the Gynecologic and Obstetric Clinic of the University of Sassari in 2013. The case concerned a Caucasian 36- year-old patient at 19 weeks and three days gestation with parity 2-012 (two vaginal deliveries in 2001 and 2005 respectively, and one miscarriage at 22 weeks). The patient did not smoke and reported to have the common childhood rashes. A family history indicated the presence of a sibling suffering from Trisomy 21.

Nuchal Translucency (NT) showed a combined risk in the standard range (1: 8071) and a value of NT of 1.1 mm. The patient underwent chorionic villus sampling which demonstrated a set of 46, XX (normal female karyotype). The diagnostic ultrasound allowed the authors to study the fetal morphology in detail. The gestational age was recalculated at 18 weeks and three days.

The authors reported multiple malformations in the upper extremities. An agenesis was estimated of the radius at level of both forearms, with agenesis of the first fingers in the hands. The humerus and ulna, and shoulder blade were found to be normally represented in morphology and size. There was no malformation nor anomaly at the level of the bony component of the carpus [7-9, 14]. At the cardiac level no morphological abnormality were observed. The scanning of the apical four chambers and in combination with color Doppler showed a normal left situs, no rhythm abnormalities, and normal aortic arch. Particular attention was paid to assess the presence of direct and indirect signs of atrial and ventricular septal defects that were not detected [4]. The patient expressed her desire to terminate the pregnancy. The woman underwent a cycle of vaginal prostaglandin at the end of which vaginal abortion was carried out.

The fetus (Figure 1) showed gross malformations such as lack of thumbs and bilateral syndactyly of II and III fingers of the right hand. The placenta was subjected to histological examination: weight of 170 grams and dimensions of 11×10×4 cm with a cord of 22 cm without any special pathological notes; focal hemorrhagic areas in the context of the placenta were detected.

At the cardiac level no morphological abnormality were observed. The scanning of the apical four chambers and in combination with color Doppler showed a normal left situs, no rhythm abnormalities, and normal aortic arch. Particular attention was paid to assess the presence of direct and indirect signs of atrial and ventricular septal defects that were not detected [4]. The patient expressed her desire to terminate the pregnancy. The woman underwent a cycle of vaginal prostaglandin at the end of which vaginal abortion was carried out.

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The fetus was subjected to X-ray (Figure 2) that showed a bilateral agenesis of the radius and the second toe on both ends. External examination showed the fetus pronate both hands and deflected medially associated with bilateral agenesis of the thumbs and fifth fingers spaced with respect to the other fingers. In the right hand there was also present syndactyly between II and III finger. External genitalia were female type. Internal examination in the forearm confirmed bilateral agenesis of the radius. Organs of the chest, head, abdomen, and pelvis showed no obvious macroscopic pathologies.

Discussion
Cardiac lesions, which may be present in complex forms in 17% of cases, include an atrial septal defect (30-60%) and ventricular patency of the ductus arteriosus, hypoplastic left ventricle, conduction abnormalities, and disruption of the aortic arch. Among the septal defects, the most frequently represented in 34% of cases are those

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of the secundum. Skeletal abnormalities save the lower limbs: this is because in the mutant gene interferes with the differentiation during the fourth and fifth weeks of pregnancy, when the lower limbs have not yet entered in the phase of differentiation. The anomalies of the upper limbs occur mainly at the expense of high radial and manifest as radial aplasia, triphalangeal thumb or absent, hand, shortness clavicular, radio-ulnar synostosis and marked prominence of the medial epicondyle, pectus excavatum, structural abnormalities of the scapula, the humerus and the ulna, to cases of phocomelia. Often the left side is more involved.

Patients that have a normal mental development and motor development may be compromised only in relation to the degree of involvement of the upper limb and scapular girdle. The patients have a life expectancy that depends primarily on the severity of the cardiac lesion. Though usually Holt Oram syndrome is not fatal, surgeries and aggressive therapies may be necessary.

The variable expressivity of the disease requires a careful examination of the family to determine whether the mutation is segregating (recurrence risk 50%) or recent (negligible risk). Identification of patients with minimal expression of the gene is difficult and clinical examination should include a search of electrocardiographic abnormalities, the bones of the carpus, and the study of the metacarpophalangeal profile.

Prenatal diagnosis is crucial for appropriate counseling, with the option of a termination of pregnancy. Although there are several cases [1-15] in the literature, there are few descriptions of prenatal diagnosis, precisely because of the high variability with which the syndrome occurs. Ultrasound diagnosis therefore focuses on the research of cardiac morphology (projection of the four chambers and axial) and the aortic arch, and all segments of the upper limb musculoskeletal. The diagnosis can be facilitated by a comparison of the increase in the value of NT. Differential diagnosis should be considered according to ultrasound conditions involving the radial ray defects, especially "thrombocytopenia with absent radius syndrome" also known as TAR (autosomal recessive syndrome presenting unilateral or bilateral agenesis of the radius, the normal development of the thumb and fetal thrombocytopenia), the VATER association, Trisomy 13, Trisomy 18. Platelet count by cordocentesis, karyotype analysis, the absence of radiological abnormalities ultrasound before and during the neonatal period, and the absence of cardiac anomalies, exclude the aforementioned diseases.
The present case is an example of how this syndrome may appear in completely heterogeneous, being characterized by only part of the anomalies that normally constitute it. The suspected diagnosis of this syndrome should be strongly influenced by the presence of anamnestic cases in the family of one or both parents. Diagnostic ultrasound allows to enter the fetus suffering from this syndrome to a procession of syndromes with similar abnormalities. The exclusion of individual anomalies, cordocentesis, and karyotype analysis are further means of diagnostic orientation. Currently, the research of the genetic anomaly on abortion and blood sampling of both parents was received. The data collected until now allowed diagnosis. The definitive diagnosis was only possible as a result of the molecular response to the presence of the mutation on genes TBX5 and TBX3.

References


Medical management of cesarean scar pregnancy at advanced age: case report and literature review

Ö. Birge, C. Karaca, D. Arslan, E. Kinali

Sudan Nyala Turkish Research and Training Hospital, Nyala (Sudan)

Summary

Aim: Cesarean scar pregnancy is a rare condition that is increasing in frequency parallel to the increase in cesarean section rates. The authors hereby discuss a case with cesarean scar pregnancy at advanced age that was treated with methotrexate (MTX) in Nyala Sudan Turkey Training and Research Hospital. Conclusion: Cesarean scar pregnancy is a rare type of ectopic pregnancy that is increasing in number due to the increase in cesarean deliveries. Clinical vigilance is imperative for diagnosis and treatment of this highly mortal and morbid entity.

Key words: Cesarean scar pregnancy; Ectopic pregnancy; Methotrexate.

Introduction

Gestational sac (GS) positioned along cesarean scar of the lesser uterine segment is a rare and life threatening form of ectopic pregnancy which was first described by Larsen and Solomon in 1978 [1-3]. Reported risk factors are mostly previous surgical intervention such as, cesarean section, dilatation and curettage (D&C), myomectomy, metroplasia, and hysteroscopy [4, 5]. Scar pregnancy occurs once in every 2,500 pregnancies [4, 5]. Data in the literature mainly consists of case reports due to rarity of the condition. However, owing to increasing rate of cesarean deliveries and frequent utilization of high resolution ultrasound devices have increased the frequency of this entity in the recent years [6]. Cesarean scar pregnancy is a potentially life threatening condition which can lead to uterine rupture, hemorrhage, disseminated intravascular coagulation, and eventually maternal death if left untreated [3]. There is no agreement in the literature on treatment due to rarity of the condition. Expectant treatment, D&C, local or systemic MTX, local potassium chloride application, embolization of uterine artery, laparoscopic excision, and hysterectomy were all suggested for different clinic presentations of the disease [7, 8]. Early and accurate diagnosis is essential for the utilization of various treatment options and preventing serious complications [7, 8]. In this paper, the authors discuss a six to seven week old cesarean scar ectopic pregnancy that was successfully treated with MTX thanks to early diagnosis and include a brief literature review.

Case Report

Forty-six year old woman, who gave birth to nine children by normal childbirth before, underwent cesarean delivery owing to a failed labor one year earlier, admitted to the Obstetrics and Gynecology Department of Nyala Sudan Training and Research Hospital in Nyala (Sudan) with two weeks menstrual delay, vaginal bleeding, and abdominal and pelvic pain. Physical examination revealed generalized pain in the inferior abdominal quadrants and speculum inspection showed a closed multipara collium and oozing bleeding possibly from the uterine cavity. Uterus size was consistent with six-week pregnancy and adnexal structures were normal. Transvaginal ultrasound imaging revealed that ovaries were normal and uterine cavity was empty with ten-mm thick tri-laminar appearing endometrium covering it. However a 22 x 30 mm gestational sac consistent with six to seven week pregnancy was detected in the junction of cervix and isthmus just adjacent to bladder, a location that fitted previous cesarean scar. Yolk sac and fetus could not be clearly identified (Figure 1). Measured beta human chorionic gonadotropin (β-HCG) level was initially 1,354 mIU/ml and the patient was hospitalized with the diagnosis of cesarean scar ectopic pregnancy for observation and treatment. Gestational sac with no fetus was measured to be 25 x 30 mm, which was consistent with seven-week pregnancy and β-HCG was 1,890 mIU/ml four days later. Patient's complaints were gradually progressing however there was no objective sign of active pelvic bleeding or rupture in transvaginal ultrasonography.

MTX treatment at 50-mg/m² dose was initiated immediately after liver and renal function tests came in normal ranges. Control measurements of β-HCG levels were obtained on the first, fourth, and seventh days. The patient was discharged on the seventh day, after β-HCG levels dropped gradually and significant relief in symptoms was observed. Weekly measurement of β-HCG levels gradually dropped to 195.56 mIU/ml, 30.76 and finally under ten mIU/ml and the patient was put to long-term follow up thereafter.
Discussion

Considering that there is an increase in frequency due to increasing number of cesarean deliveries, cesarean scar pregnancy is still one of the rarest and potentially life-threatening form of ectopic pregnancies [1, 9]. Seow et al. estimate risk of cesarean scar pregnancy as one in every 2,226 pregnancies and give rates as 0.15% among women who underwent cesarean delivery and 6.1% among women with a history of previous ectopic pregnancy [5, 10, 11]. With advancing gestational age, trophoblasts invading defective myometrium along previous cesarean scar cause abnormal vascularization in the inferior segment of the front uterine wall and eventually leading to pelvic pain and severe vaginal bleeding especially in greater gestational weeks [10]. While the most common presenting symptoms are pelvic pain and/or vaginal bleeding, the patients can be asymptomatic that usually get diagnosed during a routine examination for menstrual delay [12]. The present patient had vague symptoms that were initially menstrual delay and spotting vaginal bleeding which then progressed to severe pelvic pain over time. Differential diagnosis of cesarean scar pregnancy from cervicoisthmic pregnancies and incomplete abortion is important. Even extensive magnetic resonance imaging establishes definite diagnosis of cesarean scar pregnancy; transvaginal ultrasonography is widely used as some sonographic properties such as, 1) empty uterine cavity, 2) empty cervical canal, 3) gestational sac visualized lying along the cesarean scar between inferior segment of the uterus and bladder in sagittal views of the uterus, and 4) visualization of trophoblastic activity on color flow Doppler imaging gives valuable data for evaluating the patient [4, 10, 12]. Combination of transvaginal and transabdominal ultrasound imaging enhanced with color flow Doppler imaging can actually be used as a gold standard for diagnosis [10, 12, 13]. There is little known about the natural course of cesarean scar pregnancy. Earliest data suggest that expectant treatment is rarely successful and life threatening [10, 14]. Although expectant treatment was initially considered in the present patient, treatment plan was switched to MTX administration immediately after progression of symptoms and increase in gestational sac dimensions. Pregnancy termination in the first trimester is advised for women with cesarean scar pregnancy since there is a high risk of life threatening complications such as uterine rupture and massive bleeding [4]. There is no consensus on treatment due to rarity of the entity and little data in the literature. In a study by Shao et al., uterine artery embolization followed by curettage is reported to be superior than other therapeutic approaches [15]. Another study by Polat et al. suggests abortion for cases less than seven weeks and MTX and/or surgery for cases greater than seven weeks [16]. Similarly, in a trial of newly introduced transvaginal surgery technique, Le et al. found it superior to uterine artery embolization and chemotherapeutics [17]. Non-surgical treatment options consist of MTX (either local or systemic and single or multiple doses), uterine artery embolization and potassium chloride or hyperosmolar glucose applications. Surgical procedures are namely D&C, wedge resection of gestational sac (either by laparoscopy or laparotomy), hysterectomy, hysteroscopy, and excision of the sac [9]. Systemic administration of MTX is standard treatment for tubal and cervical pregnancies [18]. Wang et al. showed that while long term high doses of MTX or local MTX combined with intravenous MTX was required for the patients with β-HCG levels higher than 5,000 IU/ml and fetal heart beat, single dose of 100 mg or multiple divided doses (20 mg/day for five days) of MTX was sufficiently embryocidal for patients with β-HCG levels less than 5,000 IU/ml and without fetal heartbeat [19]. Although D&C carries high intraoperative bleeding risk as a primary treatment [6, 10], it can be con-
sidered as an option under the guidance of abdominal ultrasonography after MTX treatment or when serum β-HCG level drops under 50 IU/L, sub trophoblastic blood circulation is completed and there is a connection between gestational sac and uterine cavity in transvaginal ultrasonography. D&C can manage to prevent septic abortus or intermittent uterine bleeding by removing gestational sac of the cesarean scar pregnancy. D&C was also planned for the present patient after MTX treatment, but the patient refused the intervention after her complaints regressed with MTX therapy. Gestational sacs that are growing into the uterine cavity are the only true indication for D&C. According to Graesslin et al., connection formation between uterine cavity and gestational sac is a natural course of MTX treatment [20]. Serum levels of β-HCG steadily dropped following a single dose of MTX in the present patient and she was discharged uneventfully after one week when β-HCG levels returned to normal range and gestational sac was regressed in the transvaginal ultrasonography. Consequently, studies show MTX therapy alone or combined with D&C helps to avoid laparotomies and preserves fertility. However, some time is needed for β-HCG levels to drop and resolution of gestational sac [5, 19].

Conclusion

There is an increase in frequency of cesarean scar pregnancies due to increasing number of cesarean deliveries. It is a severe late complication of cesarean deliveries that can lead to loss of fertility and even death. Clinical suspicion and measurement of serum β-HCG levels along with an evaluation with transvaginal ultrasonography is crucial for early diagnosis. Delays in diagnosis and treatment of cesarean scar pregnancy can lead to life threatening conditions and prompt emergent hysterecтомies for hemostasis.

References

Urinary catheterization as a successful treatment option for post-cesarean section vesicouterine fistula

A.A. Rouzi1, N. Sahly1, N. Mansouri1, K. Khashoggi2, L. Ashkar2
Departments of Obstetrics and Gynecology1 and Radiology2, King Abdulaziz University, Jeddah (Kingdom of Saudi Arabia)

Summary
Surgery, the usual treatment option for vesicouterine fistula (VUF), is often delayed to allow involution of the uterus. The authors report a case of successful treatment with urinary catheterization. A 39-year-old, gravida 7, para 6, woman presented at term with obstructed labor. She had one previous cesarean section followed by a vaginal birth before. She underwent emergency cesarean section. She was readmitted after one week because of pelvic collection. Aspiration revealed pus and urine. Retrograde cystogram and pelvic MRI confirmed the presence of VUF. Urinary bladder catheterization for six weeks resulted in the successful treatment of the fistula. Urinary catheterization in the early postpartum period can result in resolution of post-cesarean section VUF, without delaying surgical intervention if it becomes necessary.

Key words: Urinary catheterization; Vesicouterine fistula.

Introduction
Vesicouterine fistula (VUF) is the least common genitourinary fistula, and has been reported to account for 7% to 25% of fistula cases [1, 2]. The incidence appears to be increasing with the increase in cesarean section. Surgical repair is the standard treatment. However, conservative management by inducing amenorrhea with continuous combined oral contraception has been reported as a valid option in selected cases [3]. The authors report a case of post-cesarean section VUF successfully treated with urinary catheterization only.

Case Report
A 39-year-old unregistered Somali woman gravida 7, para 6 presented to the emergency room at King Abdulaziz University Hospital, Jeddah, Saudi Arabia in labor at 40 weeks gestation. Her membranes ruptured 14 hours prior to presentation. Fetal movement had been absent for the previous two days. She had a cesarean section three years prior because of severe preeclampsia, which was followed by one successful vaginal delivery. Vaginal examination showed a five-cm dilated but edematous cervix, vertex at -2 station with marked caput, and molding. Fetal heart sounds were absent. She underwent an uneventful emergency cesarean section and delivered a male stillborn of 3.68 kg. Her postoperative course was satisfactory, and she was discharged home on the third postoperative day. One week later, she presented to the emergency room with fever and difficulty voiding. Pelvic ultrasound showed 10 x 6 cm pelvic collection behind the urinary bladder. The patient was admitted and she was started on ampicillin, gentamycin, and clindamycin. She underwent CT-guided aspiration of the pelvic collection. Pus and urine were drained. Retrograde cystogram showed leaking of dye from the bladder and a fistulous communication between the urinary bladder and the endometrial cavity (Figure 1). Pelvic MRI confirmed the diagnosis of VUF (Figure 2). Intravenous pyelography showed a normal upper renal tract. The fistula was treated conservatively by inserting a 16 Fr Foley catheter in the urinary bladder and leaving it in situ for six weeks. A repeat retrograde cystogram showed no leakage of the dye and absence of the vesicouterine fistula.

Discussion
Due to its infrequent occurrence, case reports and limited case series provide guidance on managing VUF. Cesarean section is the most common cause of VUF, contributing to the perceived increasing incidence concomitant with the increasing rate of cesarean section [4]. A history of multiple cesarean sections is frequently observed, and other cases with a history of successful or failed vaginal birth after cesarean have been reported. Surgery is considered the mainstay for repair of VUF, and many authors restrict a conservative approach to cases that are diagnosed early during the postoperative period [5, 6]. The involution of the uterus may promote healing during this interval. Reports of failure predominate over successes following conservative management with catheterization, and surely contribute to the acceptance of surgery as the gold standard treatment. For example, two cases managed conservatively for four and six weeks required subsequent surgical repair, which was performed laparoscopically [7]. In a series of 22 patients with VUF, seven women were first treated conservatively, with closed bladder catheterization for three weeks...
and treatment of urinary infection [8]. These patients were diagnosed “early” after cesarean section, which was defined as within the first six months postoperatively. The conservative approach was not successful, and the women subsequently underwent successful surgical repair of the fistulae, comprising open transabdominal surgery with omental flap interposition that was also used successfully in the remaining patients. Hormonal treatment, which may be combined with catheterization, has been reported to be effective in resolving VUF [3]. Healthcare practitioners should be diligent in monitoring possible signs and symptoms of VUF that may allow early diagnosis and conservative treatment. The initial evaluations in the present patient led to a high suspicion of VUF; however, even subtle presentations should arouse a suspicion of VUF in high-risk women. Symptomatic patients who are diagnosed with VUF within the postpartum window conducive to conservative management, but who did not present for medical care until symptoms became intolerable, may choose to have surgery if they are challenged by having to endure catheterization and continuing leakage while waiting for an uncertain resolution.

Using a surgical approach to repair VUF is often recommended to be delayed until at least three to six months postoperatively; however, some reports advocate early surgical intervention. Therefore, unless conditions indicate a conservative approach is contraindicated, the authors believe that catheterization is a valid option for patients during the early postoperative period. High-risk patients should be educated about symptoms of fistula and counseled to return for medical attention when they occur, to increase opportunities to follow early conservative management. While the probability of success following catheter-only management remains unclear, it is important to encourage reports of resolution following this conservative approach that includes adequate case information to help define in which patients success is most likely.

In conclusion, a trial of conservative catheter-only management of VUF presenting one-week postpartum was successful at resolving the fistula. Educating high-risk mothers about VUF symptoms promotes early diagnosis and allows a trial of conservative intervention prior to maturation of the fistulous tract.

References

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Address reprint requests to:
A.A. ROUZI, FRCSC
P.O. Box 80215
Jeddah 21589 (Kingdom of Saudi Arabia)
e-mail: aarouzi@gmail.com
Acute intestinal obstruction due to a non-involuting uterus after cesarean section: case report

K. Karaman¹, M. Ercan¹, H. Demir², M. Yener Uzunoglu², S. Bostanci³

¹ Sakarya University, Faculty of Medicine, Department of General Surgery, Sakarya
² Sakarya Teaching and Research Hospital, Department of General Surgery, Sakarya
³ Sakarya Teaching and Research Hospital, Department of Obstetrics and Gynecology, Sakarya (Turkey)

Summary
The involution of the uterus is influenced by a number of factors such as advanced childbearing age, electrolyte disturbances, multiparity, repeated cesarean sections, and vaginal infections. The authors report the management of a clinical case of a 41-year-old female who presented with acute intestinal obstruction due to a non-involuting uterus after cesarean section.

Key words: Non-involuting uterus; Intestinal obstruction; Cesarean section.

Introduction
Reasons for acute intestinal obstruction after cesarean sections are usually related to formation of adhesions [1]. Although rare, acute pseudo-colonic obstruction (Ogilvie’s syndrome) has also been reported after cesarean sections [2]. The authors report the management of a clinical case of a 41-year-old female who presented with acute intestinal obstruction due to a non-involuting uterus after cesarean section.

Case Report
A 41-year-old female was referred for intestinal obstruction from an obstetric center on the first postpartum day after a successful delivery by cesarean section. This was the fourth live birth and her first cesarean section after three vaginal births. Indication for cesarean section was due to delay over one week in delivery after 40 weeks of pregnancy and fetal distress. Physical examination revealed abdominal distention with rebound tenderness. Her white blood cell count was 15,000/mm³. Plain abdominal graphics showed dilated multiple small and large bowel segments (Figures 1A and B). Abdominal computerized tomography showed a large non-involuting uterus measuring approximately 20 cm in diameter accompanying dilated small and large bowel segments which had been thought as an acute abdominal compartment syndrome requiring immediate decompression (Figure 1C). Thus, the patient underwent an emergent abdominal laparotomy. During exploration, all small bowel segments and the colon were diffusely dilated from Treitz ligament to recto-sigmoid region. The recto-sigmoid colon was compressed between the uterus and sacral promontorium, which was the cause of the mechanic obstruction (Figure 1D). A hole was performed to the transverse colon to relieve distention by milking gas and bowel fluid. After intraoperative consultation to obstetric and gynecology department for hysterectomy, it was decided to leave the uterus in situ because of legal reasons and fears that the patient may want further pregnancies in the future. Additionally, intravenous oxytocin and methylergonovine maleate were applied for uterine involution. However, little response was observed. The hole in the transverse colon could be closed by primary repair, but a transverse loop colostomy was performed instead, with the attempt to control damage while performing involution of the uterus and compression of uterus on recto-sigmoid colon that persisted throughout this process. Patient’s postoperative course was uneventful and she was discharged on the fifth postoperative day. Two months later, a successful colostomy closure was performed.

Discussion
There are some risks of complications after cesarean section including adhesions, infections and wound complications, bleeding, bowel injury and obstruction, hysterectomy, extended operative time and hospital stay, and delays in delivery. Intestinal obstructions after cesarean sections are related usually to adhesions, which can develop after infection, surgery, and chemical irritation. The normal wound-healing process after injury to the peritoneum involves a complex inflammatory cascade of fibrin deposition, coagulation, and influx of inflammatory cells resulting in band and adhesion formation [3]. Another cause of intestinal obstruction after cesarean section, although rare, is acute pseudo-colonic obstruction known as Ogilvie’s syndrome in which dilatation of large bowel segments occurs in the absence of a mechanic obstruction [4]. However the disease is seen mainly in patients who have co-existing medical problems (diabetes mellitus, major depression), and immobility [5]. Drugs (opioids, tocolytics, etc) and electrolyte disturbances may also be responsible [6]. However, primary mechanism of acute intestinal obstruction in the present case was compression of a non-involuting uterus on recto-sigmoid colon. After research of the English literature, this is the second reported case [7].
The involution of the uterus is influenced by a number of factors such as age, abnormalities associated with calving (dystocia, retained fetal membranes, hypocalcemia, ketosis, twin births, and metritis), and a delayed return to normal cyclic activity in the ovaries [8]. Multiparity is another factor in delaying uterine involution which is explained by the increased uterine size [9-11]. In the clinical study by Al-Bassam, it was found that uterine involution is delayed in women delivered newborns weighing more than four kg, and in women who with high vaginal swab show presence of pathogenic organism. Further, uterine involution was faster in women delivered vaginally compared to those delivered by emergency cesarean section regardless to the weight of the newborn [12]. Dimitrow et al. observed a slower and unsteady uterine involution after cesarean section, which was more prominent after re-sections [13]. The mean reason of a non-involuted uterus in the present case could not be elucidated. The infant's born weight was 3.5 kg, which is considered in normal range, and this was her first cesarean section. Neither electrolyte deficiencies such as hypomagnesemia or hypocalcemia nor vaginal infection were detected. Multiparity and advanced childbearing age may be contributed factors for an atonic uterus leading to delay in involution of uterus in this case.

In conclusion, although uncommon, a non-involuted uterus can lead in the early postoperative period to acute intestinal obstruction by compressing the recto-sigmoid colon.

References
Acute intestinal obstruction due to a non-involuting uterus after cesarean section: case report


Address reprint requests to:
K. KARAMAN, M.D.
Arabacılı mah Eski Kazımpaşa Cad No:76
Atioglu Sitesi B Blok Kapısı Girisi Daire: 4
Serdivan, 54055, Sakarya (Turkey)
e-mail: karaman_kerem@yahoo.com.tr
Correlation of serum albumin with the clinical features and prognosis of preterm neonates in the neonatal intensive care unit

C.Y. Yang, B.Y. Li, P. Xu, Y.J. Yang, Q.Z. Yang

Department of Paediatrics, Liaocheng People’s Hospital (Liaocheng Clinical School of Taishan Medical University), Liaocheng (China)

Summary

Objective: To evaluate the clinical significance of serum albumin (ALB) levels in the early evaluation and prognosis of preterm infants in the neonatal intensive care units (NICUs). Materials and Methods: The authors collected and retrospectively analyzed complete clinical records of preterm infants admitted to the NICU from July 2012 to March 2013. The cases were divided into three groups according to their ALB levels: ≥30 g/L, 25–30 g/L, and ≤25 g/L. Results The mean gestational age in the ≤25 g/L ALB group was significantly higher than that in the ≥30 g/L ALB group ([33.41 ± 2.15] weeks) (p < 0.05). The prealbumin, blood platelet, and blood urea nitrogen in the ≤25 g/L ALB group were significantly lower than those in the > 30 g/L ALB group (p < 0.05). In addition, serum lactate in the ≤25 g/L ALB group was significantly higher than that in the ≥30 g/L ALB group (p < 0.05). Conclusion Serum ALB level increased with increasing gestational age. Lower ALB levels were associated with more perinatal complications, damage to multiple organs, more severe cases, and mechanical ventilation, which resulted in longer hospital stays and poorer prognoses.

Key words: Preterm infant; Serum albumin; Clinical performance; Prognosis.

Introduction

Advances in perinatal care have improved the survival rate of very low birth weight (VLBW) preterm neonates, and even extremely low birth weight (ELBW) preterm neonates, as well as the high morbidity of severe infections. Hepatic protein synthesis in preterm neonates is less than that in full-term neonates; thus, serum protein levels are indicators for evaluating fetal and preterm protein nutrition. Serum albumin (ALB) is a negative acute-phase protein; thus, the degree of hypoalbuminemia of critically ill patients is correlated with the intensity of the inflammatory response triggered by infections [1]. Nevertheless, ALB levels should be considered an indicator for severity and a reliable indicator for frailty, a physiological condition characterized by low functional reserve, high susceptibility to stressors, and unstable homeostasis. However, research on protein levels and clinically relevant indicators in preterm neonates are limited. The whole clinical data regarding the preterm neonates were analyzed retrospectively from the cases admitted in the present neonate intensive care unit (NICU) between July 2012 and March 2013. The authors explored the clinical significance of different ALB levels during the early assessment and prognosis of preterm neonates to provide a basis for clinical therapy and medication. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Liaocheng Peoples Hospital. Written informed consent was obtained from all participants.

Materials and Methods

Patient selection

The preterm neonates were recruited in the retrospective observational study were admitted into the present NICU from July 2012 to March 2013. The inclusion criteria were as follows: 1) gestational age < 37 weeks; 2) the patients were admitted within 24 hours; and 3) the patients did not receive any serum or blood products before the blood samples were collected. The exclusion criteria were as follows: 1) age > 24 hours when blood was collected; 2) maternal and fetal blood antigen groups were incompatible; and the mother received plasmapheresis during pregnancy; 3) neonates with associated congenital malformations, chromosomal disorders, and suspected genetic metabolic diseases; and 4) incomplete clinical data.

Methods

All subjects underwent routine physical examination and laboratory tests after admission. An additional sample was drawn from the same arterial sampling point using lithium heparin vacutainer tubes. The samples were sent to the central laboratory of the present hospital. Plasma and serum were subjected to multicomponent analyses. Laboratory tests: arterial and venous blood samples were collected within 24 hours after birth. The following laboratory variables were determined: serum albumin (ALB), prealbumin (PA), urea nitrogen (BUN), creatinine (Cr), creatine kinase (CK), isoenzyme (CKMB), high-sensitivity C-reactive protein (CRP), white blood cell, blood platelets, lactate (Lac), and so on. The other indicators included neonatal critical illness score (NCIS): the most abnormal values within 24 hours were considered the first score. The authors also recorded gestational age, birth weight, sex, mode of delivery, duration of hospitalization, complications, prognosis, and other complications during pregnancy.

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The study was approved by the medical ethics committee of the hospital. All newborns who were admitted into the NICU were divided into three groups according to the ALB level: ≥ 30 g/L, 25 g/L to 30 g/L, and ≤ 25 g/L.

Statistical analysis
The data are presented as the mean ± standard deviation (SD) if they followed a normal distribution. Differences among groups were tested using an ANOVA. The number of cases and percentages were used for the count data, and R was determined using two chi-square tests. However, if the data did not meet the requirements of the chi-square test, a Fisher exact test was performed. Differences with \( p < 0.05 \) were considered statistically significant.

Results

General information
A total of 339 preterm neonates were recruited into the study: 201 boys and 138 girls; eight cases were twins and one case involved triplets, 135 deliveries were natural childbirth and 204 were via cesarean section. The subjects were divided into three groups: ≤ 25 g/L (168 cases), 25 g/L to 30 g/L (87 cases), and ≥ 30 g/L (84 cases). The mean ALB level for the ≤ 25 g/L group was \((23.11 \pm 1.90)\) g/L, that for the 25 g/L to 30 g/L group was \((27.45 \pm 1.18)\) g/L, and that for the ≥ 30 g/L group it was \((31.93 \pm 2.26)\) g/L.

The mean gestational age for the 25 g/L to 30 g/L group was \((32.47 \pm 2.22)\) weeks and that for the ≥30 g/L group was \((33.41 \pm 2.15)\) weeks \((p < 0.05)\). The three groups did not significantly differ in terms of birth weight \((p = 0.753\); Table 1).
Severe infections, organ damage, NCIS (n=339).

<table>
<thead>
<tr>
<th>Severe infection, n.</th>
<th>≤25 g/L</th>
<th>25-30 g/L</th>
<th>≥30 g/L</th>
<th>F or X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ damage, n.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4</td>
<td>135</td>
<td>66</td>
<td>69</td>
<td>0.371</td>
<td>0.691</td>
</tr>
<tr>
<td>≥4</td>
<td>33</td>
<td>21</td>
<td>15</td>
<td>12.000</td>
<td>0.035</td>
</tr>
<tr>
<td>NCIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual, n.</td>
<td>108</td>
<td>36</td>
<td>39</td>
<td>4.078</td>
<td>0.037</td>
</tr>
<tr>
<td>Non-individual, mean ± SD</td>
<td>94.70±6.10</td>
<td>95.41±4.94</td>
<td>97.07±3.92</td>
<td>0.917</td>
<td>0.407</td>
</tr>
<tr>
<td>Mechanical ventilation (invasive/non-invasive)</td>
<td>39</td>
<td>12</td>
<td>16</td>
<td>3.299</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Outcomes and length of stay (n=339).

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>≤25 g/L</th>
<th>25-30 g/L</th>
<th>≥30 g/L</th>
<th>F or X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival, n.</td>
<td>150</td>
<td>87</td>
<td>84</td>
<td>4.885</td>
<td>0.027</td>
</tr>
<tr>
<td>Death, n.</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of stay, mean ± SD, d</td>
<td>24.38±11.17</td>
<td>22.51±13.31</td>
<td>17.07±10.24</td>
<td>3.002</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Severe infection complications, organ damage, and NCIS

The incidence rates of severe infection did not significantly differ among the three groups (17.9%, 11.5%, and 10.7%, p > 0.05). Up to 33 patients in the ≤ 25 g/L ALB group, 21 patients in the 25 g/L to 30 g/L, and 15 cases in the ≥ 30 g/L ALB group developed functional damage to ≥ four organs (F = 12.000, P = 0.035). A total of 108 cases (64.3%) in the ≤25 g/L ALB group, 36 cases (41.4%) in the 25 g/L to 30 g/L ALB group, and 39 cases (46.4%) in the ≥30 g/L ALB group had single critical illness scores (sugar levels less than 1.0 mmol/L) (χ² = 4.078, P = 0.037; Table 4).

Outcomes and length of stay

The length of stay in each group was (24.38 ± 11.17), (22.51±13.31), and (17.07 ± 10.24) days, respectively (F = 3.002, p = 0.049). The ≤ 25 g/L ALB group had 12 deaths, whereas the other two groups had no deaths (χ² = 4.885, p = 0.027; Table 5).

Discussion

ALB is the most abundant plasma protein and it is synthesized exclusively in the liver. ALB maintains the colloid osmotic pressure of the blood (accounting for 80%), acts as a buffer, and it transports bilirubin, uremic toxins, porphyrins, fatty acids, metals, cortisol, thyroxine, endotoxins, medications, and endogenous nitric oxide. Furthermore, ALB may be an important antioxidant; thus, it may contribute to neuronal survival during development. These functions are of vital importance to critically ill preterm neonates [2]. Human ALB 4% prolongs the survival of endotoxemic mice. Human ALB 4% activates endothelial nitric oxide synthase and restores lipopoly-saccharide-impaired flow-dependent endothelial dilation of mesenteric arteries. This finding is associated with downregulation of nuclear factor κB and upregulation of nuclear respiratory factor-2 and heme oxygenase-1 [3]. Low ALB levels are common in critically ill patients, with incidences rates as high as 40% to 50% [4]. In a study with a large number of critically ill preterm neonates, Iacobelli et al. [5] reported that hypoproteinemia (total protein levels less than 40 g/L) on day 1 of life is an independent factor associated with severe adverse outcome (SAO), defined as in-hospital death or severe neurologic injury on cranial ultrasound). Thus, the present authors designed the study to explore the clinical significance of different ALB levels on the early assessment and prognosis of preterm neonates to provide a basis for clinical therapy and medication.

The premature infants were delivered preterm because of various reasons, and have incomplete liver development, especially in VLBW and ELBW neonates. Preterm neonates have lower levels of ALB and prealbumin because of decreased synthesis and inadequate reserves [6-8]. In recent years, more studies have evaluated fetal nutrition. Arteto et al. suggested that albumin levels are closely related to the severity of the disease and prognosis. However, ALB infusion has not been investigated clinically [9]. Therefore, understanding the actual serum protein levels and clinically relevant indicators will help us better assess clinical conditions of patients to provide a reliable basis for drug therapy.

The average gestational age was (32.47 ± 2.22) weeks in the ≤ 25 g/L ALB group and (33.41 ± 2.15) weeks in the ≥ 30 g/L ALB group (p < 0.05). Up to 36 cases (21.4%) in the ≤ 25 ALB group, 18 cases (20.7%) in the 25 g/L to 30 g/L ALB group, and 42 cases (50%) in the > 30 g/L ALB group developed pregnancy-induced hypertension. The present authors found that low economic level and poor awareness of antenatal care resulted in more obstetric diseases; for example severe gestational hypertension and preeclampsia (70%). Severe pre-eclampsia causes significant mortality and morbidity to both the mother and the child [10]. Organ damage is common during early preeclampsia, especially to the placenta and the liver. The reduced ALB synthesis after IUGR reflects low intrauterine ALB synthesis because of the lower availability of amino acids in utero. The data suggest that a low supply of intrauterine nutrients restricts the growth and potentially reduces the postnatal protein turnover, including ALB turnover [11-13]. The mean weight of the neonates in the ≤ 25 g/L ALB group ≥ 25 g/L was (1.68 ± 0.46) kg and that in the > 30 g/L ALB group was (2.07 ± 0.48) kg (p = 0.000).

Sharma et al. [14] found platelet count is negatively correlated with the incidence of neonatal sepsis. Torkaman et al. [15] et al. showed that thrombocytopenia is negatively correlated with the incidence of neonatal sepsis. In the present study, the platelet count in the ≤ 25 g/L ALB group was...
lower than those in the other two groups (223.02 ± 85.19, 247.42 ± 58.34, 264.79 ± 77.45; p < 0.05). In the ≤ 25 g/L ALB group, 30 cases developed premature rupture of membranes (PROM) (17.9%), which indicates that ALB level is an indirect indicator for inflammation. Thus, we need to observe the associated clinical manifestations to control the infection in time [1]. BUN and creatinine are indicators of renal function and indirect indicators of nutritional state. In the present study, the BUN level was higher in the > 30 g/L ALB group than in the ≤ 25 g/L ALB group. However, the renal blood flow and glomerular filtration rate after birth increased rapidly with decreasing renal vascular resistance and increased systemic blood pressure. Thus, we should observe clinical changes to diagnose the pathology promptly.

Blood Lac level is a sensitive biochemical marker for tissue perfusion and oxygen delivery that can be used to assess disease severity and outcome. Some studies have shown that blood Lac levels are negatively correlated with the neonatal critical illness score [16-19]. In the present study, the Lac level was (4.60 ± 3.19) mmol/L in the ≤ 25 g/L ALB group, which is higher than the (3.38 ± 2.29) mmol/L in the ≥ 30 g/L ALB group. Therefore, ALB level and serum Lac complement each other when used for evaluating the condition of preterm neonates [20-22].

NCIS is commonly used in the clinical assessment of neonates. The scores accurately reflect the severity of illness, with lower scores indicating higher chances of the mortality [23-25]. In the ≤ 25 g/L ALB group, 108 cases were subjected to single NCIS (64.3%) and 39 cases required mechanical ventilation [invasive and non-invasive mechanical ventilation (23.2%)]. Logistic multivariate analysis revealed that NCIS and mechanical ventilatory support are independent risk factors for neonatal outcome. However, non-invasive mechanical ventilation, NCPAP, and NIPPV may be preferred for clinical assisted ventilation.

The study by Vincent et al. [26] indicated that the severity of disease increases by 89% and the mortality rate increases by 137% when ALB is as low as 10 g/L. Higher mortality rates were also correlated with damage to more than four organs. In the present study, 33 cases (19.6%) in the ≤ 25 g/L ALB group developed damage to at least four organs, which is significantly higher than in the other groups. Twelve patients died in the ≤ 25 g/L ALB group, whereas no patients died in the other two groups (p < 0.05). The mean length stay was (24.38 ± 11.17) days in the ≤ 25 g/L ALB group, which is longer than the (24.38±11.17) days in the ≥ 30 g/L ALB group. Furthermore, the cost of hospitalization was high, which aggravated the economic burden.

Iacobelli et al. showed that low plasma protein concentrations within the first day of life is strongly predictive of a negative outcome in VLBW [5]. Logistic and multiple regression analysis confirmed that the association of hypoproteinemia-SAO remained significant after adjusting for other major predictors of outcome at baseline (odds ratio, 3.4; 95% confidence interval, 2.1–5.4; p < 0.0001). Hypoproteinemia was highly associated with SAO in this cohort of critically ill preterm infants [5]. The increased capillary permeability in critical cases was sufficient to sequester ALB into the interstitium. Vincent et al. showed that hypoalbuminemia at discharge is correlated with ICU readmission and unexpected deaths [26].

Therefore, searching for new indicators in sick preterm infants should include markers that can be used to assess the condition. In conclusion, ALB is a sensitive indicator for predicting the early condition and prognosis of preterm neonates. The present analysis only included a small sample size. Therefore, further studies are needed to understand the actual ALB levels, particularly in a large sample of preterm neonates, especially VLBW and ELBW. Moreover, understanding the connection between ALB and prognosis is important for determining whether human blood ALB should be administered intravenously.

References


Correlation of serum albumin with the clinical features and prognosis of preterm neonates in the neonatal intensive care unit


Address reprint requests to:
P. XU, M.D.
Department of Paediatrics
Liaocheng Peoples Hospital
Dong Chang Xi Road, No. 67
Liaocheng 252000 (China)
e-mail: pingxuencn@126.com
Combination of selected biochemical markers and cervical length in the prediction of impending preterm delivery in symptomatic patients

M. Hadži-Lega¹, A. Daneva Markova¹, M. Stefanovic², M. Tanturovski¹

¹ University Clinic of Obstetrics and Gynecology, Medical Faculty, Ss. Cyril and Methodius University, Skopje (Republic of Macedonia)
² Departments of Obstetrics and Gynecology, Medical Faculty Nis (Republic of Serbia)

Summary
The pathophysiology of preterm delivery (PTD) is complex and multifactorial. It occurs in 8–12% of all deliveries, and the rate of PTD has increased during the past years in spite of intensive efforts towards early detection and prompt treatment. Fifty-eight pregnant women were eligible to join the study if they attended the University Clinic for Gynecology and Obstetrics, Skopje and were admitted to Department of High Risk pregnancy Unit with symptoms of preterm labor (PTL) (symptoms of uterine activity judged by the assessing physician to be indicative of PTL) at 24.0 to 36.6 weeks gestation. Test specimens for fetal fibronectin (fFN), phosphorylated insulin like growth factor binding protein 1 (phIGFBP-1), IL-6, and IL-2R and measuring the cervical length via transvaginal ultrasound were performed for each patient. The best statistical model for predicting PTL in the present study was to use a combination of the phIGFBP-1 test, a positive fFN test, cervical length less than 21.5mm, levels of IL-6 higher than 1,305 pg/ml in the cervico-vaginal fluid (CVF), and serum levels of C-reactive protein (CRP) higher than 6.1mg/L which was excellent at identifying the patients that were to deliver within 14 days of admittance.

Key words: Preterm delivery; Fetal fibronectin; Cytokines; Predictors; ph-IGFBP-1.

Introduction
Prevention of preterm delivery (PTD) is a major obstetrical challenge. This is not only due to assisted conception, as increased PTD rates have been demonstrated also among spontaneous pregnancies. The pathophysiology is complex and multifactorial. The rate of preterm births has been estimated at around 14.9 million, which accounts for 11.1% of all live births worldwide[1]. Individual countries incidence rates are highly dependent on the degree of development and range from 5% in most developed European countries to 18% in several African countries[1]. It occurs in 8–12% of all deliveries, and the rate of preterm delivery has increased during the past years in spite of intensive efforts towards early detection and prompt treatment [2]. Currently, cervico-vaginal fetal fibronectin (fFN) test and trans-vaginal ultrasound measurement of cervix length are recommended by the American Congress of Obstetricians and Gynecologists for the prediction of PTD [2]. However, there is an urgent need for new markers that may be easier and more sensitive than current methods because the majority of women who had undergone a transvaginal ultrasound examination and pelvic examination can experience discomfort [3].

Fetal and neonatal morbidity and mortality rates are strongly associated with gestational age at birth. Specifically, infants born before 32 weeks of gestation are at risk of sequela [4-6]. More than 70% of women presenting with symptoms of preterm labor (PTL) do not progress to active labor and delivery [7, 8]. It is essential to identify pregnant women with threatened PTL who will deliver preterm, and differentiate them from the women who will continue their pregnancies to full term. The past three decades have seen a plethora of attempts at developing methods to correctly predict preterm delivery, such as obstetric history, symptoms, epidemiological risk factors, maternal indicators such as age and anthropometric parameters, pregnancy characteristics such as bleeding, different physical examination parameters and biological markers etc., but most of these methods are neither sensitive nor specific enough [9, 10]. Any method potent enough to accurately distinguish women who are likely to deliver preterm infants from those who have the symptoms and clinical presentation, but are unlikely to delivery prematurely, would certainly go a long way towards preventing unnecessary and potentially risky medical interventions and reduce costs and burden to the medical system.

Attempts to predict PTD based on maternal and biochemical data, and interventions to reduce PTD rates, have been largely unsuccessful [11–13]. The necessity of finding
reliable prediction models is urgent, and a multiple-markers test indicative of the multifactorial etiology of PTD is likely to be more successful [14, 15]. There is a wealth of literature suggesting that cervical length measured by ultrasound and fFN have the potential to improve the prediction of PTD [16-18]. In order to institute specific therapy more appropriately, it is important to have adjunct tests to help predict who is most likely to deliver preterm. The detection of phosphorylated insulin like growth factor binding protein 1 (phIGFBP-1) in the cervical secretions of women presenting with PTL has been shown to be associated with an increased risk of PTD [19-28]. Recently, cervico-vaginal concentrations of phIGFBP-1 have been shown to correlate with the risk of PTD [19-28]. Disruption to the chorio-decidual interface results in elevated levels in cervical secretions. Potentially contaminating body fluids with fFN—such as semen and urine—contain only trace quantities of phIGFBP-1 [19].

Another group of biochemical markers involved in the prediction of PTD is inflammatory molecules such as cytokines. Cytokines may be involved in the etiology of preterm birth through their influence on prostaglandin synthesis and secretion [29]. A number of studies have reported increased concentrations of certain cytokines, most notably interleukin 6 (IL-6) in the serum and amniotic fluid of patients with PTL [30-34]. Several studies have investigated IL-6 detection in the cervico-vaginal fluid (CVF) and demonstrated that the presence of IL-6 in the CVF is associated with PTD [35-37].

Increased serum concentrations of the C-reactive protein (CRP) in the first trimester do not increase the overall risk of PTD [38], but in symptomatic patients’ serum CRP levels have a low sensitivity (38%) and high specificity (94%) in the prediction of PTD before 34 weeks of gestation [39].

Increased serum levels of the soluble interleukin 6 receptor (IL-6R) have been associated with development of preeclampsia and eclampsia, as well as PTD and intrauterine growth restriction [40].

Most of these markers can be used in conjunction with the evaluation of the cervical length via transvaginal ultrasound. Studies conducted in the general population of pregnant women have shown that the decrease in cervical length is a predictor of PTD, although it has a low predictive value (6-47.6%) [41-45].

However, threatened PTD and PTD requiring treatment at high-level medical facilities are increasing in Macedonia. In these circumstances, to extract unknown factors related to preterm delivery, the authors carried out a study using patients diagnosed with threatened preterm delivery admitted to the Department of high risk pregnancy at University Clinic of Obstetrics and Gynecology Skopje, which is a tertiary medical organization and they combined different independent predictive factors of premature delivery.

The aim of this study was to evaluate the usefulness of different biochemical markers, namely: fFN, phIGFBP-1, IL-6, IL-2-R, and CRP, in the prediction of preterm delivery within 14 days of admission in symptomatic patients, as well as the predictive and diagnostic value of the combination of markers along with the cervical length evaluated via transvaginal ultrasonography. The hypothesis of the study was that the combination of markers would yield better results in predicting PTD within 14 days of admission than each marker.

Material and Methods

Fifty-eight pregnant women were eligible to join the study if they attended the University Clinic for Gynecology and Obstetrics, Skopje and were admitted to Department of High Risk pregnancy Unit with symptoms of preterm labor (symptoms of uterine activity judged by the assessing physician to be indicative of PTL) at 24.0 to 36.6 weeks gestation. They were recruited in period of six months from September 2013 to March 2014. They were with symptoms or complaints suggestive of PTL including uterine contractions, intermittent lower abdominal pain, and pelvic pressure. Recruited patients had intact amniotic membranes determined by speculum examination and minimal cervical dilatation (≤ 3 cm). Women were excluded if they had ruptured membranes, antepartum hemorrhage, active labor, a cervical cerclage in place, and suspected chorioamnionitis (defined by fever, abdominal pain, leukocytosis).

Consenting women were treated according to usual hospital protocol. The authors took a detailed history, performed tocography, and a speculum exam to obtain test specimens for fFN, phIGFBP-1, IL-6, IL-2R and drew blood to determine the adequate serum concentrations of the respective markers and measured the cervical length via transvaginal ultrasound.

The obtained data was digitized, and all statistical tests were performed using SPSS version 13.0. The authors used descriptive statistical analysis to display the following parameters: mean, standard deviation, coefficient of variation, and interval of variation. The categorical variables were tested using Chi square and Fischer exact tests, and the quantitative variables were analyzed with the independent sample test and Mann-Whitney’s U test. To determine the correlation between the variables, the authors used Spearman Rank Ordered Correlation test and Pearson’s coefficient of linear correlation. The also used binary logistic regression to determine the predictive role of the analyzed parameters in the prediction of preterm labor. Receiver operating characteristic (ROC) curves and the area under the curve (AUC) were computed for each biochemical markers and cervical length.

Results

The main demographic characteristics of the study population are shown in Table 1. Mean maternal age was 30.12 years. Mean gestational age was 31.55 weeks at recruitment. Mean height was 164.34 cm. Mean weight was 74.05 kg. Mean BMI was 27.54. Of the 58 patients enrolled in the study, nine had history of previous PTD. The authors also evaluated the number of previous spontaneous abortions, parity, and smoking of the patients with threatened preterm labor.

Thirty-six patients (62.07%) delivered within 14 days from admission. Table 2 presents the distribution of the patients that delivered or remained pregnant within 14 days of admission in regards to the results of the fFN. From the 36
patients that were delivered within 14 days of admission, 27 patients (75%) had a positive fFN test, while 15 patients (68.18%) of the 22 patients that remained pregnant after 14 days from admission had a negative fFN test. The Chi-square statistical test confirmed that this observed difference was statistically significant ($p = 0.0011$).

The fFN test is a significant predictor of preterm delivery. Patients with a positive fFN test have an OR of 6.429 (95% CI 1.991 - 20.758) to deliver prematurely. The diagnostic performance of the fFN test in the present study was as follows: sensitivity = 75%; specificity = 68.2%; PPV = 79%; NPV = 62.5%; positive likelihood ratio (LR+) = 2.46; negative likelihood ratio (LR-) = 0.37; AUC = 0.716; 95% CI = 0.575-0.856. Figure 1 shows the ROC curve for the diagnostic performance of the fFN test.

In the group of patients that delivered within 14 days of admission, the authors found significantly higher concentrations of IL-6 in the CVF ($p = 0.0011$). The average measured concentration of IL-6 was 3,139.8 ± 2,646.2 pg/ml in the group of patients that delivered within 14 days, while the average concentration in the group that remained pregnant longer than 14 days was 1,755.7 ± 3,165.7 pg/ml. Using the data, the authors calculated a ROC curve in order to determine the cut-off value for the concentration of IL-6 in the CVF that accurately predicts preterm delivery (Figure 2). The results determined that the best cut-off value for the concentration of IL-6 in the CVF that correctly predicted preterm delivery in this study was 1,305 pg/ml, which gave the test a sensitivity of 69.4%, specificity of 68.2%, a LR+ of 2.18 and a LR- of 0.45. The calculated rate of PTD was 78.13% in patients with a concentration of IL-6 in the CVF higher than 1,305 pg/ml, and 42.31% in the patients with concentrations lower than the cut-off.

The difference in the concentration of IL-6 in the CVF, classified above or below the determined cut-off of 1,305 pg/ml, between the two groups of patients was statistically significant ($p = 0.005$)

### Table 1. — Demographic characteristics of study population (n=58).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ±SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>30.12 ± 4.82 (20 - 40)</td>
</tr>
<tr>
<td>Gestation age at examination (w)</td>
<td>31.55 ± 3.95 (22 - 36)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.54 ± 4.93 (18.7 - 43.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parity</th>
<th>n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>13 (22.41)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>45 (77.59)</td>
</tr>
</tbody>
</table>

| Previous preterm delivery | 10 (17.24) |
| Smoker | 11 (18.96) |

### Table 2.— Detection of fetal fibronectin in the CVF of the studied population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Outcome within 14 days of admission</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undelivered (n=22)</td>
<td>Delivered (n=36)</td>
</tr>
<tr>
<td>Fetal fibronectin (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>7 (31.82%)</td>
<td>27 (75.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>15 (68.18%)</td>
<td>9 (25.0%)</td>
</tr>
</tbody>
</table>

Figure 1.— ROC curve for the performance of the fFN test in the prediction of preterm delivery.

Figure 2.— ROC curve for the performance of IL-6 in the CVF as a predictor of preterm delivery.
The patients that gave birth within 14 days of admission were also statistically more likely to have a positive phIGFBP-1 test \((p = 0.02)\). The distribution of patients with regards to the phIGFBP-1 test is shown in Table 3.

All but one pregnant women that remained pregnant after 14 days of admission had a serum level of IL-2R below 500 U/ml and the difference in concentrations between the two groups was statistically significant \((p = 0.044, \text{Table 4})\).

The authors calculated an optimal cut-off value for the IL-2R levels of 388.5 pg/ml, which yielded a sensitivity of 69.4%, specificity of 68.2%, LR+ 2.18, LR- 0.45, and an AUC of 0.688 (Figure 3).

The group of patients that were delivered within 14 days of admission had significantly higher serum levels of CRP, when compared to the patients that remained pregnant after 14 days \((p = 0.001)\). The average CRP concentration in the PTD group was 11.9 ± 16.85 mg/l versus 5.67 ± 5.5 mg/l in the group of patients that remained pregnant after two weeks.

The optimal cut-off value for the CRP serum concentrations that correctly predicted PTD in this group of patients was 6.1 mg/l, which gave the test a sensitivity of 72.2%, specificity of 72.7%, LR+ 2.64, LR- 0.38, and an AUC of 0.756 (Figure 4).

The patients that were delivered within 14 days of admission in the present study group had an average cervical length of 18.78 ± 5.8 mm, which was significantly lower than the average cervical length \((23.87 ± 6.36 \text{ mm})\) of patients that remained pregnant after 14 days \((p = 0.0028)\). The shortest cervical lengths that the authors measured in the two groups were five and 12 mm, respectively. The univariate logistic regression revealed that the best cut-off value for the cervical length in the present study was 21.5 mm, which yielded a sensitivity of 30.6% and a specificity of 36.4%. The ROC curve for the diagnostic performance of the transvaginally measured cervical length is shown in Figure 5.

Table 5 summarizes the diagnostic performance of each individual test.

**Combination of markers**

The authors used multivariate logistic regression to devise models based on the combination of different tests. Table 6 summarizes the diagnostic performance of each tested combination. The combination of cervical length (measured transvaginally) of less than 21.5, positive fFN and phIGFBP-1 tests, as well as serum concentrations of

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**Table 3. — Detection of phIGFBP-1 in the CVF of the studied population.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Outcome within 14 days of admission</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undelivered ((n=22))</td>
<td>Delivered ((n=36))</td>
</tr>
<tr>
<td>PhIGFBP-1 n (%)</td>
<td>Positive</td>
<td>14 (63.64%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8 (36.36%)</td>
</tr>
</tbody>
</table>

**Table 4. — Serum levels of the soluble IL-2R in the studied population.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Outcome within 14 days of admission</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undelivered ((n=22))</td>
<td>Delivered ((n=36))</td>
</tr>
<tr>
<td>Serum concentration of IL-2R (U/ml) (\text{range 169-748})</td>
<td>382.8±138.6</td>
<td>471±197.6</td>
</tr>
<tr>
<td></td>
<td>(\text{range 199-1327})</td>
<td>(\text{range 199-1327})</td>
</tr>
</tbody>
</table>

Figure 3.— ROC curve for the performance of IL-2R serum concentration as a predictor of preterm delivery.

Figure 4.— ROC curve for the performance of CRP serum concentration as a predictor of preterm delivery.
Combination of selected biochemical markers and cervical length in the prediction of impending preterm delivery in symptomatic patients

CRP and IL-2R and CVF concentration of IL-6 above their respective cut-off values yielded the best calculated probability form PTD within 14 days of admission ($p = 0.995$).

Figure 6 demonstrates the ROC curves of the individual combinations. The combination of the six markers has an AUC of 0.912 (95% CI 0.837 - 0.987) which indicates that this particular combination of predictive markers makes a precise classification of cases of PTL into two groups of patients: one in which the patients are very likely to deliver within 14 days of admission and another in which the patients are very likely to remain pregnant after 14 days.

Discussion

Despite advances in obstetric care, PTD remains a major cause of neonatal morbidity and mortality. With women presenting an acute risk of PTD, tocolysis, steroids, and in utero transfer to a center with neonatal intensive care are recommended [46]. This involves unnecessary treatment and complex management in a relevant number of symptomatic women who eventually will not deliver preterm. Therefore, there is a need for assessment tools to reliably identify cases who are at highest risk of early delivery, and those who are not and can avoid treatment.

This fact clearly illustrates the necessity of devising a model that correctly predicts imminent PTD. Given the multifactorial etiology of PTD as a syndrome, it is safe to assume that predictive models that utilize multiple specific markers have a better chance of succeeding.

There have been a limited number of studies that investigated the relationship between different biochemical markers in the maternal serum and CVF and the occurrence of PTD. One such study [17] published a predictive model based on the serum and CVF inflammatory markers in the early second trimester (12-25 weeks of gestation) in asymp-
tomatic patients with a previous PTD. Their model correctly predicted 69% of the recurrent PTDs. Holst et al. [8] conducted a study on 89 patients and analyzed 27 protein markers associated with PTD in the amniotic fluid and CVF and devised a prediction model based on amniotic macrophage inflammatory protein-1 beta, cervical interferon-gamma, and monocyte chemoattractant protein-1. The authors demonstrated that their model correctly identified the symptomatic patients that would deliver prematurely within seven days of testing.

Transvaginal ultrasonographic cervical length measurement is a commonly used method of evaluating patients with symptoms of PTL. The generally accepted cut-off value of 25 mm or less is considered to be a relevant predictor of an impending PTD in patients with symptoms of PTL [47-49]. The patients that delivered within 14 days of admission in the present study group had an average cervical length of 18.78 ± 5.8 mm and the optimal cut-off value for the cervical length in this study was 21.5 mm, which yielded a sensitivity of 30.6% and a specificity of 36.4%.

Previous studies also demonstrated that patients with PTL and clinical chorioamnionitis have elevated concentrations of IL-6 in the amniotic fluid and umbilical cord blood serum [22-27]. A recent large study conducted by Woodworth et al. [28] focused on the diagnostic accuracy of IL-6 detected in the CVF as a predictor to PTD. The authors analysed 660 CVF samples for IL-6 and concluded that the IL-6 test with a cut-off of 250 pg/ml had a sensitivity of 35%, specificity 87%, PPV 19%, NPV 98%, LR+ of 4.83, and LR- of 0.41. These results over-perform the test in the present analysis. The present regression analysis gave a significantly higher cut-off value for the concentration of IL-6, as opposed to the now almost universally-accepted value of 250 pg/ml, first determined by Lockwood et al. [9]. This may be due to the fact that the authors calculated the likelihood of delivery within 14 days as opposed to seven days, the small sample size, and the fact that the study recruited a high-risk group of patients that already had symptoms of PTL, a high proportion of which (over 60%) delivered within 14 days.

A multitude of published studies undoubtedly demonstrated the clinical relevance of fFN testing for the assessment of patients at risk of PTD. One of the more relevant such studies was a double-blinded study that evaluated the use of fFN in patients with threatened PTL [29]. The study enrolled 763 patients and used a cut-off of 50 ng/ml. The calculated NPV for delivery within 14 days of admission was 99.2%. For patients that tested positive for fFN, the authors calculated the risk of delivery at 38.8%, although the PPV was only 13.4%. Most authors agree that the greatest value for fFN testing in symptomatic preterm patients is its high NPV with the potential to reduce unnecessary intervention.

Conclusion

The best statistical model for predicting PTL in the present study was to use a combination of the phIFGBP-1 test, a positive fFN test, cervical length less than 21.5 mm, levels of IL-6 higher than 1,305 pg/ml in the CVF, and serum levels of CRP higher than 6.1 mg/l which was excellent at identifying the patients that were to deliver within 14 days of admittance.

The combination of these tests performed better than each individual test in the present population and decreased the false positive rate, which in turn reduced the chances for inappropriate patient treatment, bringing down the costs. Still, the present study was hindered by a small sample size and burdened by recruiting only high-risk symptomatic patients which influenced the results.

The study is only the beginning of this type of research in the present population. Further research is required in terms of the evaluation of cost-benefits of using such tests to prevent subsequent unnecessary interventions in the low-risk group, as well as achieve the benefits from such intervention in the high-risk groups of patients.

References

Combination of selected biochemical markers and cervical length in the prediction of impending preterm delivery in symptomatic patients


Severe antenatal strangulation and sudden fetal death occurs in term: case report

D.B. Mian¹, J. Konan¹, K.C. Kouakou², V. Angoi¹, E. Gbary¹, C. Itoua¹

¹ Department of Gynecology and Obstetrics of Cocody, Abidjan
² Department of Pediatric of Cocody, Abidjan (Republic of Cote d’Ivoire)
³ Department of Gynecology and Obstetrics of Brazzaville, Brazzaville (Congo)

Summary
The authors report a case of a sudden antenatal death, by severe strangulation, unlikely related in a term pregnancy; multiple loops of nuchal umbilical cord (UC) (ten), rarely describe in literature, were observed around the fetal neck. The in utero fetal death (IFD) was suspected by the non-attendance of fetal movements and confirmed by US scan. The tight nuchal cord around the neck (tCAN) diagnostic was made during caesarean delivery, as it was not discovered in pregnancy US scan monitoring nor in the US scan made in emergency. The newborn examination shows severe fetal strangulation by the presence of many spires of a too long UC (1.50 m). Autopsy was not been accepted by the family. Through this reported case the authors wanted to show the difficulties of its diagnosis in less developed Sub-Saharan country were US scan practice is not usual.

Key words: Antenatal fetal death; Multiple loops; Nuchal cord; Strangulation.

Introduction
Intrauterine fetal death (IFD) is a devastating complication of pregnancy, an exceptional painful event from whom any parent is prepared. The overall incidence of stillbirth is reported to be around 1.2% with an incidence after 20 weeks of 1%, after 28 weeks of 0.4% and at term of 0.2% [1]. The underlying reasons can be divided in maternal, fetal, and placental causes. Concerning the fetus, umbilical cord (UC) complications seem to be the most common cause for fetal demise in the third trimester [2]. Multiple nuchal cord loops of UC around the neck (CAN) are common with an incidence reported to be between 15.8% and 30% [3]. Unfortunately these complications are regarded as unpredictable and unpreventable [4] Tight nuchal umbilical CANs (Figure 1) are relatively frequent findings and some times associated with a negative fetal outcome. During pregnancy, strangulation is exceptional (Figure 2) but can occur at anytime during second or third trimesters. Strangulation and constriction of UC can be associated and lead to acute intrauterine asphyxia. Fetal deaths occurred frequently (15-30 %) during trial [4].

Herein clinical case the authors report a sudden IFD in third trimester, due to a severe strangulation of tight umbilical cord around neck (tCAN) with multiple UC turns. Through it the authors would like to show difficulties of it diagnosis and management in low socio-economic African countries.

Case Report
A 16-year-old primiparous, with no particular individual antecedents, was admitted for a broken right femur by firearm. She was carrying a progressive pregnancy estimated at 39 weeks. The investigation found no pelvic pain or vaginal discharge, and fetal active movements were well felt by the patient. Hemodynamic and general state of health were good. Obstetrical examination found a soft uterus height measuring 32 cm. Fetal heart beats were regular at fetal Doppler at 148 beats / minute. Fetal presentation was vertical but higher. X-ray radiography found a closed break of right femur. Ultrasound scan found a fetus in breech presentation with regular cardiac activity regular. No diagnosis of nuchal cord (NC) was evoked. Placent was well adhered and had a normal insertion, with no placental hematoma. A cesarean section had been planned and right leg was placed in splints awaiting for surgery. Fetal movement assessment and fetal Doppler were performed twice in the day. The clinical course was satisfactory but three days later the patient indicated difficulties in perceiving fetal movement without any other signs. Fetal Doppler showed no fetal heart activity, confirmed by US scan. Cesarean section was performed and showed a stillborn with intraoperative multiple torsions of UC around the neck, with severe entanglement (Figure 2). The stillborn had weight of 3,100 grams and did not present any external abnormalities or dysmorphogenic signs (Figure 1). The UC had a length of 143 cm and contained three blood vessels (Figure 3). No constriction, knots or any others abnormalities (cupula nor abruptio placentae) were found (Figure 3). No histological examination of placenta and autopsy were done. Treatment of broken femur was performed in the same surgical procedure. The postoperative course was simple with healing of cesarean wound section observed two weeks later. The remaining of the follow-up was made by a trauma specialist.


Discussion

NC is defined as loop of UC 360° or more around the fetal neck. “Tight” is defined as the inability to manually reduce the loop over the fetal head, and loosen as the ability to manual reduce the loop over the head. [5]. In the present case, the authors found multiple NC loops which is a very uncommon finding.

According to Lal Neena et al., NC come and go during gestation, but become more frequent towards term [6]. It is most frequently seen among UC abnormalities and the prevalence has been reported to be 15-24% at delivery [6-8]. The incidence increases with advancing gestation from 12% at 24 to 26 weeks to 37% at term [9]. They are not associated with perinatal morbidity and mortality but in some fetuses and newborns, CAN may cause problems, especially when the cord is tightly wrapped around the neck (Figure 1). The cluster of cardiorespiratory and neurological signs and symptoms associated with unique physical features that occur secondary to tight cord around-the-neck has been referred to as tCAN syndrome [9].

Few studies have shown that NC and or tCAN can affect the outcome of delivery and may have long-term effects on the infant [10]. Meanwhile, their role as risk factor of stillbirth is always discussed [10, 11] and this reported case proved the contrary. However, some case reports of post-mortem findings on stillbirths show negative pathology reports and the present authors agree that tCAN is the only cause of death [12]. CAN represent 15-30% of deliveries and are observed enough during labour [13] and responsible for about 50% of fetal cardiac beat anomalies [14]. It is the unique physical features of tCAN syndrome that distinguishes it from birth asphyxia even though there are many similarities. According to Bendon et al. [15], UC abnormalities are considered as one of the causative factors for birth asphyxia and NCs can be sub-classified by the number of nuchal wrapping, by complex wrapping of other body parts, and by the type of loop [15]. In type A we have la simple 360° rotation that must end with the placental end of cord on top, versus type B in which the fetus rotates through the loop creating an incipient knot with the fetal end of the cord. Other studies classified tCAN (those cut to enable delivery) versus loose cords. In general the more complicated the nuchal wrapping, the more likely that there will be complications [15].

The clinical significance of NC is controversial. Many factors contributed to the controversy such as tightness of the NC, cords wrapped around the neck in a “locked fashion” or not, and multiple cord entanglements around the fetal neck [3]. It is suggested that a moderate tight CAN would impair cephalic venous blood flow only, whereas a very tight would compromise the umbilical circulation and produce systemic hypoxia, hypercapnia, and academia [3].

During pregnancy, the indentations of the neck with NC can be deep, but there is little evidence that the cord causes
significant tracheal obstruction. A few case reports suggest that the cord interfered with the fetal swallowing and caused polyhydramnios [16]. In ICAN, there may be jugular venous obstruction especially if the NC tightens one [17]. The presence of NC alone is not a cause of stillbirth. In an otherwise unexplained fetal death, assigning cord wrapping as cause of stillbirth is supported by finding a complicated wrapping or a very tight cord as evidence of tension. A short length of free cord from the neck to placenta or from the neck to the umbilicus would support cord compression or torsion as mechanism of death. If the cord is still attached to the infant, the type A or B of wrapping can be determined simply by which segment of cord is on top (cephalad). Type B may be more likely to result in cord occlusion [15].

During labour, the manifestation of tCAN symptomatology seems to occur both in the presence of normal and depressed Apgar scores [18]. UC strangulation due to tCAN may cause obstruction of blood flow first in thin umbilical vein wall, while infant’s blood continues to be pumped out through the thicker walled umbilical arteries, thus causing hypovolemia and hypotension resulting in acidosis [19]. Anemia [18] and mild respiratory distress may occur. Case of facial and conjunctival petechiae [19] and rarely petechiae of the neck and upper part of the chest and skin abrasion of neck (Figure 2), have been described [20] where the cord was tightly wrapped and facial suffusion [21], as in the described case. Some born alive infants may also be somewhat obtunded with a low tone and have transient feeding difficulties. These findings raise the possibility of transient encephalopathy, which may lead to long-term complications [9]. A stillbirth attributed to a cord problem should have evidence of cord obstruction or circulatory compromise. Other potential causes of stillbirth need to be excluded prior to labelling cord abnormalities as causative factor, since they be seen in more than a third of all normal live births [9].

tCAN syndrome may conceptually be considered as strangulation which may result in non-lethal problems or death as in the present observation. The pathophysiological mechanisms of strangulation injuries (lethal and non-lethal) involves venous, arterial obstruction (arterial spasm due to carotid pressure) in the neck and vaginal collapse (increased parasympathetic tone) [9]. This can lead to cerebral stagnation, hypoxia, and unconsciousness and at last loss of muscle tone. A study on potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight showed evidence of association of tCAN in children with quadriplegia [22].

Rocha et al. showed that intermittent UC occlusion in preterm and near term sheep, caused a decline in pO2 and pH, and higher PCO2 and altered brain protein synthesis/degradation [12]. According to Parast et al., significant correlation of placental changes of “minimal histologic criteria” are associated with cord accidents (as tCAN is part of cord accidents), using specific placental histologic criteria by restriction of umbilical blood flow in unexplained stillbirth [10].

With CAN, the UC measured over 70 cm (6-7%) or more than one meter for others (1%). In a series of 104 cases with CAN (7.5%), Gasser et al. [23] found macerated fetus received at the laboratory by showing obviously a constriction of the neck or a limb, or fetal body by a stretched cord, which is abnormally too long. The excess length of UC, as explained, can be risk factor of this funicular disease. Kecksztein et al. [24] found that this risk can be multiplied when UC is longer than 70 cm.

The diagnosis of CAN is possible during pregnancy but not simple, particularly in undeveloped countries. Indeed US scan machines use is not readily available. The best moment for the present research was third trimester of pregnancy. Color Doppler Ultrasound is a good investigation when it detects the presence of a NC if the UC could be followed 360 degrees around the fetal neck [25], with a sensitivity in diagnosing of 37.5%, specificity of 80%, and positive and negative predictive values respectively estimated at 29% and 85%, according to Peregrine et al. [26]. In poor countries, US scan is rarely employed during pregnancy, firstly for its high cost and secondly for its unavailability in most maternities. Otherwise, many pregnant in poor countries are illiterate and came to maternities only when they are ready to deliver. CAN diagnostic is mostly utilized during labour or at birth in poor countries. According to Peregrine et al., the sensitivity of the US diagnosis of a NC is low prior to induction of labor at term. Therefore NC does not appear to be important for many authors in choosing of way of birth. The low US detection rate of a NC may limit its use in decision of induction of labour in high-risk pregnancies [26]; however, during labour, diagnosis may be suspected in front of abnormal fetal heart rate (FHR) occurring during a uterine contraction, or a lack of progression of the presentation. The immediate threat of CAN presence is fetal distress which presents during labour via two types of changes: an alarming slowdown of FHR (detected by monitoring) during the first contractions or altering of amniotic fluid after membranes rupture. These two problems require acceleration of delivery either by classical vaginal delivery with systematic episiotomy or by performing a cesarean section. Cesarean section planning is recommended as mode of delivery in the present center because of impossibility of fetal monitoring during labor, besides the too long of a delay to perform a caesarean section.

Antenatal or perinatal fetal death represents a tragedy or negative experience sometimes incomprehensible regarded as failure of pregnancy monitoring. This kind of death has variable frequency according to socio-economic level of population. This frequency is low in developed countries (4% to 10%), and high in poor countries (15% to 50%). When umbilical cord is coiled around the neck, it accounts for 70% to 80% of all umbilical cord complications noticed at deliveries. For World Health Organization there was 4.1% of in utero fetal death in Africa and 37% of these deaths occur during labour [27]. Fetal death etiologies are multiple and varied and can be caused by fetal, adnexal or maternal reasons. Nowa-
days, tCAN is increasingly blamed in the adverse issue of these pregnancies. Indeed tCAN can cause fetal asphyxia by strangulation even if there are no uterine contractions. In the current case, there were no uterine contractions but the authors observed many loops around neck, which explain the sudden onset of fetal death by severe strangulation. In literature, tCAN is often related to decreased placental oxygen transfer with signs of fetal acute hypoxia by fetal perfusion disorders. The coiled cord, while blocking blood circulation, induces placental parenchymal infarction, edema, congestion, and / or fucicular thrombosis. No maternal disease was diagnosed prenatally. In case a fetal anemia occurs systematically at birth due to fetal-maternal hemorrhage can also be observed. It may be necessary to cut UC between two clips before performing the release of fetal shoulders in deliveries [28].

Newborn with a tCAN were slightly more likely to be admitted to neonatal intensive care unit (6.6% vs. 5.9% admission rate, p = 0.000). The subset of very low birth weight neonates with a CAN, compared with those with no NC, were of the same gestational age and birth weight, with same Apgar scores, and were not more likely to have severe intraventricular hemorrhage, retinopathy of prematurity or periventricular leukomalacia, or to die. NC in normal pregnancies at term associated with an increased rate of caesarean delivery in nul- lar leukomalacia, or to die. NC in normal pregnancies at term may be caused by multiple nuchal cord loops. [Ultrasound Obstet. Gynecol., 2010, 35, 253.]


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