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Contents

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ORIGINAL ARTICLES

CASE REPORTS

Distinct types of uterine adenomyosis based on laparoscopic and histopathologic criteria

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Summary

Purpose: To analyze laparoscopically treated cases of adenomyosis based on intraoperative and histopathology findings and to correlate different types with patients' presenting symptoms and characteristics, as well as with the surgical approach. *Materials and Methods*: Sixty-eight women who underwent laparoscopic treatment of adenomyosis at a referral center for gynecological laparoscopy. *Results:* Four distinct types of adenomyosis could be identified: diffuse, sclerotic, nodular, and cystic (54.5%, 13%, 28%, and 4.5% of cases, respectively). Menorrhagia as the main presenting symptom was significantly more frequent in patients with the diffuse type (84%) compared to those with sclerotic (44%) and nodular (37%) types ($p = 0.025$ and $p = 0.001$, respectively). All cases of cystic and nodular adenomyosis were treated by laparoscopic excision of the lesion. Eighty-nine percent of patients with sclerotic adenomyosis were treated with wide laparoscopic excision of the abnormal tissue. Eighty-one percent of patients with diffuse adenomyosis were treated with laparoscopic hysterectomy. Conclusions: Adenomyosis can be classified in four distinct types with differences in the presenting symptoms, as well as in the ideal surgical approach.

Key words: Adenomyosis; Laparoscopy; Laparoscopic adenomyomectomy; Laparoscopic hysterectomy.

Introduction

Uterine adenomyosis is a rather common gynecological disorder; the precise etiology as well as the mechanisms leading to the disorder are still not clearly determined. Clinical studies have proposed that adenomyosis results when endometrial glands invade the myometrial layer [1, 2]. In addition, Bird *et al.* suggested that the diagnosis of adenomyosis requires the identification of a smooth-muscle hyperplasia reaction [1]. It is postulated that disruptions of the endometrial-myometrial border allows for a reactive hyperplasia of the endometrial basalis layer and its extension into myometrium [3, 4]. Magnetic resonance imaging (MRI) can be useful for the detection of the junctional zone thickening, as well as for the evaluation of the myometrial invasion depth [5]. To date the main histopathologic criteria of adenomyosis are the existence of myometrial hypertrophy around a focus of adenomyosis and also the distance between the adenomyotic lesion and the endo-myometrial junction which has to measure at least the 25% of the total myometrial thickness [6].

Two distinct types of the disease have been described, diffuse and focal. The diffuse type is defined by the presence of multiple foci of adenomyosis distributed within the myometrium [3]. The focal type is defined by the presence of isolated nodules of hypertrophic myometrium and ectopic endometrium, also referred as adenomyomas. The current classification of adenomyosis has not been changed for almost a century when Cullen (1908) was the first who distinguished adenomyomas and diffuse adenomyomas [7]. Recent developments in diagnostic techniques [8], along with the wide application of endoscopic minimally invasive techniques in gynecology and the increased reports of uterus-sparing surgical procedures for the treatment of uterine pathology may alter the current management strategies for adenomyosis [9].

The aim of the present study was to analyze all the laparoscopically treated cases of adenomyosis in the present department based on intraoperative and histopathology findings and to correlate different types of the disease with patients' presenting symptoms and characteristics, as well as with the surgical approach.

Materials and Methods

The present study included all patients who had laparoscopic treatment for uterine adenomyosis at the Department of Gynecology, Lefkos Stavros Hospital, Athens, between January 2005 and June 2012. Sixty-eight patients were included in the study which was approved by the relevant institutional review board. All patients had a preoperative transvaginal sonographic (TVS) evaluation and were surgically treated by the same team of surgeons. All patients were treated either by laparoscopic excision of the lesion(s) or by subtotal laparoscopic hysterectomy, according to the preoperative and intraoperative clinical estimation of the extent and type of the adenomyosis and the patient's wish for future fertility. Diagnosis of adenomyosis was confirmed by histopathological examination in all specimens.

In patients treated with laparoscopic excision of adenomyosis with uterine preservation, the laparoscope was inserted into the abdominal cavity from an 11-mm supra-umbilical incision using the open access technique [10]. Three additional trocars were introduced under direct vision. After visual inspection of the pelvic cavity, the site of the uterine incision was determined by visual palpation of the uterus and based on the previous sonographic evaluation. Diluted vasopressin was injected (one ml of 20 U of vaso-

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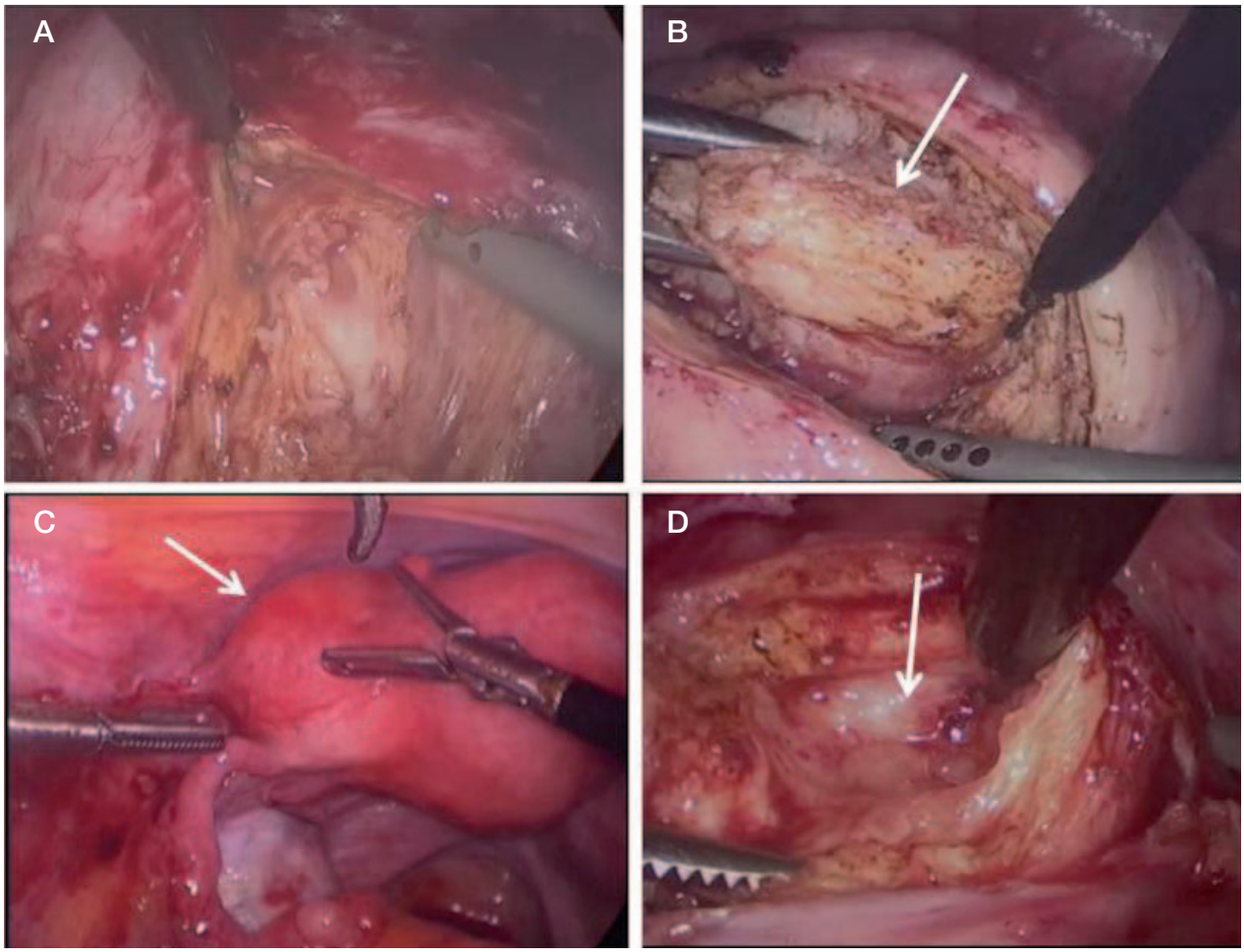

Figure 1. — Four types of adenomyosis identified during laparoscopy.
A: Diffuse. The entire myometrium has a spongiform texture.
B: Sclerotic. The lesion has an off-white fibrotic appearance (arrow).
C: Nodular. The arrow indicates a well-defined spherical lesion located on the left cornual region.
D: Cystic. The arrow indicates the base of the adenomyotic cyst.

pressin diluted with 40 ml of normal saline) in the myometrium around the affected area. The uterine wall was incised with monopolar current, set at 30 watts. The adenomyotic lesion was then isolated from the macroscopically healthy myometrium. Primary consideration was the removal of the whole adenomyosis while preserving as much unaffected myometrium as possible. Color and vascularity of the tissue was the main criterion used to identify the defective tissue. Adenomyotic tissue is paler, less vascular. and bleeds less due to fibrosis in contrast with unaffected tissue which is redder and more haemorrhagic. After resection was complete, the surgical wound was closed with deep interrupted 1-monocryl suture in one or two layers depending on the depth of the wound. The serosal layer was closed with either interrupted or continuous monocryl 0 or 2.0 sutures.

In patients treated with subtotal laparoscopic hysterectomy, the typical procedure was applied [11].

Haematoxylin and eosin stain was performed in the paraffin blocks that were obtained from the surgical specimens, in order to histologically identify the adenomyosis lesions. This was followed by Masson trichrome stain (Goldner with light green) to study the collagen and the smooth muscle fibers surrounding the adenomyosis foci (red: muscle fibers - green: collagen).

Statistical analysis was performed using the SigmaStat 2.03 software. Normality tests were performed and the data were analyzed using One Way ANOVA test, t-test, $\chi 2$ test, and the Fisher Exact Test, as appropriate. Yates Correction for continuity was performed where necessary.

Results

Depending on the macroscopic appearance and texture of the lesion(s) during laparoscopy, as well as on the respective histopathological findings, patients could be classified in four distinct types of the disease: diffuse, sclerotic, nodular, and cystic adenomyosis. In the ‘diffuse’ type (37 patients, 54.5% of study sample), the myometrium was uniformly affected and the size of the uterus was increased. During ex-

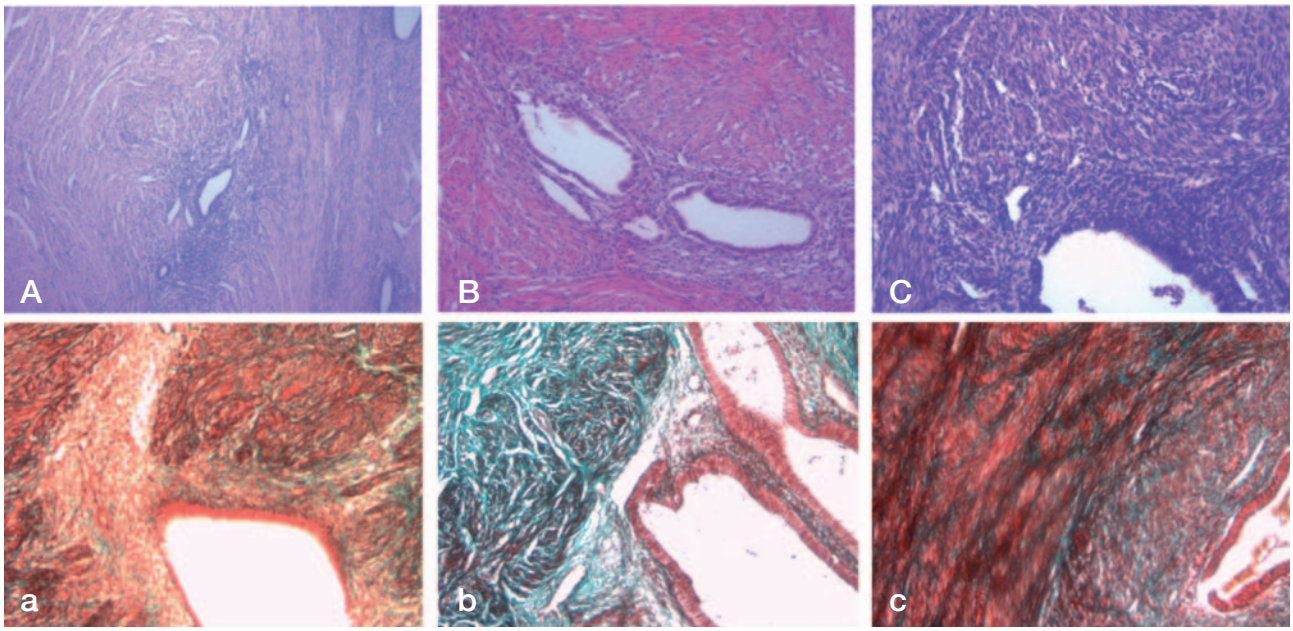

Figure 2. — Upper row represents haematoxylin and eosin stained specimens. A: Diffuse adenomyosis; an adenomyotic foci is demonstrated within normal myometrium. B: Sclerotic adenomyosis. C: Nodular adenomyosis; adenomyotic foci within leiomyomatous tissue.
Lower row represents specimens stained with the Masson trichrome histochemical stain. a: Diffuse adenomyosis; normal myometrium and smooth muscle fibers with bundled growth pattern are recognized surrounding the lesion. Scattered collagen fibers can be seen. b: Sclerotic adenomyosis; densely packed collagen fibers. C: nodular adenomyosis; densely arranged hyperplastic smooth muscle fibers; only a few collagen fibers are identified among the smooth muscle clusters.

Table 1. — *Patients' characteristics and main symptoms.*

Type	Age (yrs) Mean ± SD (Range)	Symptoms	
		Pelvic pain/ Dysmenorrhea n (%)	Menorrhagia
Diffuse	44.4 ± 6.0 (27-59)	7 (19)*	31 (84)*
Sclerotic	40.1 ± 6.4 (30-47)	5 (55)*	4 (44)*
Nodular	37.7 ± 5.2 (27-45)	13 (68)*	7 (37)*
Cystic	30.7 ± 2.5 (28-33)	2 (67)*	1 (33)*
Total	41.2 ± 6.7 (27-59)	27 (40)**	43 (63)**

*percentage of cases with the specific type of adenomyosis; **percentage of all cases of adenomyosis.

cision, the lesion had a spongiform texture (Figure 1A). Histopathology revealed multiple variable-sized foci of adenomyosis in the entire uterine wall with no hyperplasia of the smooth muscle cells. Adenomyotic lesions were spread in between normal myometrium. Smooth muscle fibers with bundled growth pattern were recognized surrounding the lesion (staining red - Figure 2A). Masson trichrome histochemical stain revealed only a few supporting collagen fibers (staining green- Figure 2a). In the 'sclerotic' type (nine patients, 13% of study sample) the lesion presented as an irregular thickening of the myometrium with an off-white pale fibrotic appearance (Figure 1B). It was firmly attached to the serosa and endometrium and, due to the hardness and friability of the tissue, grasping and suturing the tissue was difficult. Histopathology showed a segment of myometrium with multiple, variable sized foci of adenomyosis, surrounded by densely packed collagen fibers (probably as a degenerating phenomenon) with no hyperplasia of smooth muscle cells of the uterine wall (Figures 2B and 2b). In the 'nodular' type (19 patients, 28% of study sample), a spherical well defined lesion which was frequently located in the cornual region or the round ligaments was identified (Figure 1C). It represented a focus of adenomyosis with obvious hyperplasia of smooth muscle cells of the uterine wall, strangling the adenomyotic structure (probably due to reactive hyperplasia of the smooth muscle fibers (staining red, Figure 2C and 2c). Finally, in the 'cystic' type (three patients, 4.5% of study sample), the lesion had cyst characteristics, it was ≥ one cm in maximum diameter and it was independent of the uterine lumen (Figure 1D).

The characteristics and main symptoms of the study subjects are summarized in Table 1. The mean age of the patients was 41.2 years (SD: 6.7 years, min: 27 years, max: 59 years). Patients with diffuse adenomyosis (mean age: 44.4 years) were on average older than women with other types of the disease (sclerotic adenomyosis – mean age: 40.1 years, nodular adenomyosis – mean age: 37.7 years. Statistically significant difference in age was noted between patients with diffuse versus nodular adenomyosis ($p \leq 0.001$), as well as between patients with diffuse versus cystic adenomyosis ($p = 0.001$).

The main symptom in women with sclerotic and nodular type of adenomyosis was chronic pelvic pain and/or dys-

menorrhoea (55% and 68%, respectively) in comparison with only 19% of the women with the diffuse type; these differences were statistically significant ($p \leq 0.001$ and $p = 0.039$, respectively) (Table 1). On the contrary, the majority of patients (84%) with diffuse type of adenomyosis presented with menorrhagia refractory to conservative measures compared to 44% of patients with the sclerotic type ($p = 0.025$) and 37% of patients with the nodular type ($p = 0.001$).

Preoperative diagnosis of adenomyosis was established sonographically in 58 (85%) of the patients. The main indication for surgery was menorrhagia in 63% of patients, pelvic pain and/or dysmenorrhea in 40% of patients, and infertility in seven percent of patients. Diagnosis of endometriosis and fibroids had been made prior to surgery in nine and 34% of study subjects, respectively.

Laparoscopic removal of the defective adenomyotic tissue was the mode of treatment in 37 (54.5%) patients, while removal of the uterus was performed in 31 patients (45.4%). All (three) cases of cystic adenomyosis and all (19) cases of nodular adenomyosis were treated by laparoscopic removal of the adenomyotic lesion (adenomyomectomy) and reconstruction of the uterine body. Likewise, eight out of nine patients (89%) with sclerotic adenomyosis were treated with wide laparoscopic excision of the abnormal tissue and reconstruction of the uterine body. Only one patient (11%) with sclerotic disease and 30 out of 37 patients (81%) with diffuse adenomyosis underwent subtotal laparoscopic hysterectomy. No major complications occurred during or immediately after surgery.

Fibroids co-existed in 32.3% of the patients, more frequently in women with sclerotic (44%) and nodular (37%) adenomyosis than in those with diffuse adenomyosis (27%) and cystic (33%) adenomyosis, although the differences were not statistically significant. Concomitant endometriosis was present in 12% of patients with adenomyosis with no significant differences between the four types of the disease.

Discussion

Analyzing the laparoscopically treated cases of adenomyosis in the present study, on the basis of intraoperative and histopathology findings, four distinct types of the disease could be identified: diffuse, sclerotic, nodular, and cystic adenomyosis.

To date adenomyosis has been classified based on the distribution of the adenomyotic foci in the uterus [7]. When the lesions are spreading throughout the myometrium forming a large boggy uterus then adenomyosis is traditionally called ‘diffuse’. Adenomyomas are well-localized intramyometrial tumor-like lesions that resemble fibroids. This classification exists for almost a century with only addition the sporadic cases of cystic adenomyosis that are primarily affecting girls and young women [12, 13].

Dealing with several cases of traditionally called diffuse adenomyosis, the present authors have noticed distinct differences in the location, appearance, and texture of the adenomyotic tissue. There were cases where the entire myometrium was affected and the uterus had a balloon-like appearance. During dissection, the defective tissue was soft with a friable spongiform texture. The authors classified these women as having diffuse adenomyosis. In other cases, the disease was affecting only a segment of the myometrium with the rest of the uterus having a macroscopic healthy appearance. The defective tissue was hard with an off-white fibrotic appearance and was firmly attached to the serosa and endometrium. Although this type has also been described as focal or segmental adenomyosis [14], the authors referred to this type of adenomyosis as sclerotic adenomyosis. The diffused type affected the entire organ had no operative planes, therefore it was impossible to be resected. In contrast the sclerotic type, even though, its surgical margins were irregular, they could be identified due to the change of texture, color, and vascularity of the tissue. In retrospect these two groups of patients presented with different symptoms and, as expected, a wide resection of the disease with preservation of the uterus was feasible in cases of sclerotic adenomyosis. Based on the authors’ observations and according to the literature, they have included two additional types of adenomyosis, the so called “nodular adenomyosis” which refers to the traditionally called adenomyomas, and the “cystic adenomyosis” which represents the less frequent entity.

Differences in macroscopic appearance and tissue rigidity that were noticed during laparoscopy were subsequently confirmed by the histopathologic examination. In cases of diffuse adenomyosis the defective tissue was scattered along the entire myometrium. Only a few supporting collagen fibers were evident, which is a finding that partially explains the soft texture of the tissue. Nodular adenomyosis, on the other hand, presented as a rigid whitish tumor often resembling a fibroid. Densely packed hyperplastic smooth muscle fibers generated this leiomyomatous texture. The main histopathologic finding that distinguished sclerotic adenomyosis from the other two types of the disease was the high concentration of collagen fibers adjacent to the adenomyotic foci (Figure 3C). Based on the present clinical observation, there was an ascending degree of fibrosis from diffuse, to sclerotic, to nodular adenomyosis. This is partly explained by the smooth muscle hyperplasia which caused severe fibrosis in cases of nodular adenomyosis and the tightly arranged collagen fibers which resulted in a less prominent fibrotic appearance. Excess fibrosis may lead to the scarring, chronic pain and alteration of tissue function that are the characteristics of the disease [15].

Although a large proportion of patients with adenomyosis are asymptomatic [16], the disorder is known to be associated with menorrhagia and dysmenorrhea, which are both frequent indications for hysterectomy [17]. However these symptoms can be encountered in other associated pathologies, such as fibroids, endometriosis, and endometrial polyps and are considered non specific for diagnosis [18, 19]. In the present cohort, menorrhagia was the most common complaint, presenting in 63% of the cases, which is consistent with what has been reported in earlier studies

[3]. It has been hypothesized that the adenomyotic uterus is unable to contract properly during menses, resulting in increased blood loss [3]. According to that theory, diffuse adenomyosis which affects the entire uterine musculature is more likely to present with menorrhagia than focal adenomyosis. The present results indicated that menorrhagia was indeed the chief complaint in cases that were classified as diffuse. On the contrary in women with nodular, sclerotic, and cystic adenomyosis, pelvic pain and dysmenorrhea were the predominant symptoms. Therefore the authors suggest that adenomyotic lesions involving a part of the uterine body such as nodular, sclerotic. and cystic adenomyosis and also those that have a more dense and fibrotic consistency, such as nodular and sclerotic types, are more frequently associated with dysmenorrhea and pelvic pain compared to diffuse adenomyosis. The resemblance of nodular lesions with endometriotic rectovaginal nodules that has already been described enhances the relationship between this type of adenomyosis and pain [20]. This finding correlates with the already reported relation of pain with disruption of tissue architecture caused by fibrosis in cases of deep endometriosis [21].

The authors had a definite preoperative sonographic diagnosis in 85% of their patients which is in accordance with the positive predictive value (PPV) of TVS diagnosing adenomyosis reported in previous studies [22, 23]. TVS is usually the first choice of image modality when investigating cases of menorrhagia or pelvic pain, but accurate diagnosis of adenomyosis is not always possible, since correct recognition of the specific sonographic features of the disease is difficult [24, 25]. MRI is less observer dependent and thus considered a more accurate noninvasive technique for diagnosing adenomyosis [25]. Since gynecologists rely mainly on sonography to investigate a pathology, adenomyosis might be undiagnosed until after hysterectomy [26]. Moreover, up to 80% of adenomyotic uteri are associated with other benign proliferative conditions, such as endometriosis and fibroids [6], making preoperative diagnosis often problematic. Contribution of each disease to the symptomatology is difficult due to the similarity of symptoms in these conditions [16] and also due to the fact that adenomyosis is typically diagnosed only at the time of hysterectomy [19]. Concomitant adenomyosis in hysterectomy specimens of women with fibroids ranges from 15% to 57% [19, 27-29] and adenomyosis coexists with endometriosis in 28% of women or less [1, 30]. There were 22 (32.3%) women with fibroids and eight (12%) with endometriosis in the present study group. No correlation was made in the frequency of these conditions among different types of adenomyosis.

The choice of a less invasive surgical treatment in adenomyotic cases depends not only on the patient's wish for uterine preservation, but also on the surgical skills of the gynaecologist. Preoperative assessment of the topography and extent of the adenomyotic lesions is important. Because of limitations regarding visualization of the extent and location of adenomyosis, it is difficult to determine the feasibility and accuracy of complete excision when conserving the uterus. This is one of the main reasons why hysterectomy has been both the primary diagnostic and the therapeutic strategy for uterine adenomyosis [31]. Conservative surgery can be proposed in the majority of patients; however the current classification of adenomyosis is often limiting the surgical options to hysterectomy, especially in cases of diffuse adenomyosis. Advances in imaging technology and guided biopsy procedures [32], as well the increased demand of women in their forties for fertility preservation, allow and necessitate a less invasive form of treatment than hysterectomy. Uterine preservation was feasible in 54.5% of the presented patients. Diffuse adenomyosis accounted for the majority of patients that underwent a hysterectomy (30 out of 31 women). Laparoscopic resection of adenomyosis requires high expertise due to difficulty in recognizing the healthy margins of the tissue. It is essential to obtain an accurate preoperative diagnosis in order to refer the patient in a specialized center if necessary. Preoperative imaging and also change in appearance, vascularity or consistency of the tissue can guide the resection. Accurate preoperative diagnosis is essential.

Partial excision of adenomyosis during laparoscopy or laparotomy is an accepted mode of treatment in cases of cystic adenomyosis and adenomyomas [13, 33-35]. New operative techniques have been described and evaluated in relation to their feasibility and applicability [33, 36, 37]. In cases of diffuse adenomyosis however, hysterectomy is generally considered the only option. the authors consider sclerotic adenomyosis as an intermediate type of adenomyosis between diffuse and nodular which can be treated conservatively. They were able to do so in eight out of nine patients with sclerotic adenomyosis.

The main limitation of this study was the relatively small number of included patients; however, the present study may serve as a motivation for further research allowing for an updated and more detailed adenomyosis classification.

Conclusion

Contrary to the traditional two-type classification, adenomyosis can be actually classified in four distinct types (diffuse, sclerotic, nodular, and cystic) with significant differences in the presenting symptoms, as well as in the ideal surgical approach. Menorrhagia as the main presenting symptom is significantly more frequent in patients with the diffuse type compared to those with sclerotic and nodular types. All patients with cystic and nodular adenomyosis, as well as the majority of patients with the sclerotic type can be treated by laparoscopic excision of the lesion. The majority of patients with diffuse adenomyosis require treatment with laparoscopic hysterectomy.

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Microwave endometrial ablation for hypermenorrhea treatment: a new era in Japan

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Summary

An estimated six million women in Japan suffer from excessive menstruation and the treatment of this disorder has been undergoing dramatic changes recently. In April 2012, microwave endometrial ablation (MEA) was approved for insurance coverage as a K863-3: a hysteroscopic endometrial ablation (17,810 points). Since the introduction of MEA to Shimane University Hospital in August 2007, authors (KN, KM) have performed the procedure in 96 patients with excessive menstruation. They have also evaluated its safety and its efficacy, not only by comparing it to the existing surgical treatment but by quantifying patients' satisfaction levels and symptom improvement. The authors conclude that MEA is a safe, effective, a low-cost treatment, and they recommend that it be considered as a standard treatment for conservative therapy-resistant excessive menstruation.

Key words: Endometrial ablation; Menorrhagia; Insurance coverage.

Introduction

An estimated six million women in Japan suffer from excessive menstruation and the treatment of this disorder has been recently undergoing dramatic changes. In April 2012, microwave endometrial ablation (MEA) was approved for insurance coverage as a K863-3: a hysteroscopic endometrial ablation (17,810 points). To note, this newly established criteria includes MEA and hysteroscopic transcervical resection of the endometrium (TCRE), which some facilities have been performing without insurance coverage.

MEA at 9.2 GHz was developed as an alternative to hysterectomy in 1995; it uses microwaves to necrotize the endometrium, thereby controlling excessive menstruation.

Microwaves are a type of electromagnetic radiation with wavelengths of 100 μm to one m and frequencies of 300 MHz to three THz. Their most well-known use is in microwave ovens. In Japan, a microwave tissue coagulator which generates microwaves at a frequency of 2.45 GHz received manufacturing approval about 30 years ago and has been used in surgery for several kinds of tumors, including liver, kidney, and prostate cancers, to control bleeding or to necrotize a malignant tumor and its neighborhood. MEA is a novel method of endometrial ablation that incorporates a Microwave tissue coagulator. With this device, the endometrium is safely coagulated and thoroughly destroyed. In the first-generation endometrial ablation devices, a high frequency wavelength or a laser was used under hysteroscopic visualization. In the second-generation devices, high frequency waves or microwaves are administered via an intrauterine introducer, without the need for hysteroscopy.

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One of authors (YK) developed a unique microwave applicator for intrauterine use, called Sounding Applicator, in 2001. Since the tip of this applicator is thin and curved, it is able to be used for both functional excessive menstruation and for bleeding caused by uterine myomata and adenomyosis [1]. Because of its striking effectiveness, it was approved by the Ministry of Health and Labor of Japan as an advanced medical treatment in December 2008. It was approved by the Ministry of Health and Labor of Japan for insurance coverage surprisingly quickly, in April 2012.

Materials and Methods

In cooperation with one of authors (YK) and others, "Practice Guideline of MEA" were published in 2008 and revised in April 2012 [2]. According to the guidelines, MEA may be used in: 1) women who are considering hysterectomy or other surgical management of excessive menstruation; 2) women in whom conservative treatment for excessive menstrual bleeding has failed; 3) women who do not desire future fertility; 4) women who do not desire future fertility but wish to avoid hysterectomy; 5) women in whom endometrial malignancy has been excluded; 6) women in whom a sounding applicator can reach all parts of the endometrium – as long as this criterion is met, the technique can be used in women with uterine enlargement or deformity due to myomata or adenomyosis; 7) women in whom the myometrium is at least one cm in thickness throughout.

In addition, women who are not eligible for surgical treatment due to comorbidities, women who suffer from serious anemia due to hematological disease, and women with chronic renal failure, even on dialysis, are considered eligible for MEA to control excessive menstruation.

Results

Since the introduction of MEA to Shimane University Hospital in August 2007, the authors have performed the

procedure in 96 patients with excessive menstruation. Authors (KN, KM) have evaluated its safety and its efficacy, not only by comparing it to the existing surgical treatment but by quantifying patients' satisfaction levels and symptom improvement [3]. They conclude that MEA is a safe, effective, low-cost treatment and we recommend that it be considered as a standard treatment for conservative therapy-resistant excessive menstruation. MEA is also safe and effective for emergency treatment. Authors (KN, KM) obtained good results with emergency MEA in a patient with hemorrhagic shock due to excessive menstruation [4].

Theoretically, microwaves denature proteins without tissue carbonization, acting directly on water molecules to generate heat. Because it does not carbonize, MEA is more effective in stopping bleeding than electric surgical knives. The authors were able to demonstrate the safety and efficacy of MEA to control not only excess menstruation but also uterine hemorrhage during transcervical surgery for large myomata and endometrium polyps. The efficacy of MEA for massive uterine hemorrhage has been added to "Guideline for Gynecological Practice in Japan, 2011," and the use of MEA is considered favorable in this situation.

Conclusion

The authors have shown that MEA is a novel therapy for conservative treatment-resistant excessive menstruation, with a good safety profile and a low monetary cost. It is also useful in women who are not good candidates for surgery.

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Survey and analysis on birth quality influence factors of 300 cases of newborns

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Summary

Purpose: Little research has been conducted to specifically identify the correlations of birth quality influence factors of newborns and hemoglobin of gravidae and puerperal with birth weight of newborns. To investigate the correlations of birth quality influence factors of newborns and hemoglobin of gravidae and puerperal with birth weight of newborns in order to provide a scientific basis for promoting health of gravidae and their newborns. *Materials and Methods:* Three hundred cases of gravidae and puerpera treated in the present hospital were randomly selected, and questionnaire survey method was used to survey their basic situations. Also, hemoglobin values in different pregnancy stages were detected. According to birth weight of newborns, gravidae were divided into several groups to compare antepartum hemoglobin levels of various groups of gravidae. In addition, logistic regression analysis was carried out for birth quality influence factors of newborns. *Results:* Logistic regression analysis result showed that birth quality influence factors of newborns included age, nutrition situation and pregnancy healthcare education of gravidae and puerpera. In addition, birth weight of newborns was positively related to antepartum hemoglobin level of gravidae ($r = 0.746$, $p < 0.01$). *Conclusions:* It was feasible for promoting smooth delivery of gravidae and puerpera, reducing incidence rate of mother and baby complications and effectively enhancing health situations of newborns to strengthen health monitoring of gravidae and conduct health education intervention.

Key words: Birth quality; Health education intervention; Influence factor; Newborn.

Introduction

Birth population quality is the basis for human healthy development, and it is the necessary condition of ensuring population quality [1]. Birth quality of newborns not only affects their own health status, but also will cause long-term and far-reaching influences to their future growth and development [2]. If birth quality of newborns cannot be effectively controlled, low birth quality population will become a huge social burden and seriously influence and restrict the sustainable development of society, economy, population, and other aspects in China. Therefore, it is a problem to be urgently solved to research the current situation of birth quality of newborns on population level under modern conditions and investigate the method of enhancing birth quality of newborns.

Birth quality of newborns is decided mainly by two factors: pregnancy week and fetal growth rate in uterus [3]. Pregnancy week decides birth weight, while fetal growth rate in uterus is possibly related to demographic characteristics, previous abnormal pregnancy history and other medical risk factors of gravidae [4]. Some studies [5] found that for gravidae with hemoglobin increase, incidence rate of low birth weight newborn apparently increased. Multiple factors can influence birth quality of newborns [6-8]. Some literatures [9,10] reported that if puerpera age was older and personality is more introversive, the possibility of pregnancy complications, gestational bacterial or viral infection, placenta abnormality, and the possibility of low birth quality of newborns increased. In addition, some literatures reported [4] that delivery mode and the time number of delivery could also influence birth quality of newborns, but the conclusions [11,12] were inconsistent.

This study carried out investigations of birth situations for 300 cases of newborns and basic situations, pregnancy nutrition and health care situations of puerperal in the present hospital and obtained influence factors of birth quality of newborns through logistic regression analysis; One-way ANOVA and LSD-t test were used to investigate the correlation between hemoglobin of gravidae and puerperal with birth weight of newborns. It lays the scientific foundation for improving health level of newborns in this region and preventing birth defects of newborns.

Materials and Methods

Objects

From January to December 2011, 300 cases of gravidae and puerperal treated in the present hospital were randomly selected. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Jinan Central Hospital. Written informed consent was also obtained from all participants. The ages were between 21 and 39 years , and the mean age was 27.32±6.57 years. Also, these gravidae had balanced ratios regarding the aspects of occupation and living standard, and they had no chronic disease and pregnancy complication. Investigated contents included basic information, disease history of, diet and life situations, etc.

**Contributed equally to this work.*

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Table 1. — *Quantification situations of birth quality influence factors of newborns.*

Factor code	Factor name	Quantification scheme
Y	birth quality of newborns	1 = giant baby 2 = normal 3 = low birth weight
X1	age	1 ≤ 24years old 2 = 25 to 30years old 3 = 31 to 35 4 = 35 to 39 years old
X2	Education degree	1 = illiteracy 2 = elementary school 3 = junior middle school 4 = High school or technical secondary school 5 = college for professional training or higher
X3	Monthly household income	1 ≤ 200Yuan 2 = 200 to 400 Yuan 3 = 400 to 600 Yuan 4 = 600 to 1000 Yuan 5 = over 1,000 Yuan
X4	Pregnancy stage	1 = 1 to 12 weeks 2 = 13 to 27 weeks 3 = 28 weeks to antepartum
X5	drug administration history	0 = none 1 = yes
X6	Nutrition situation	1 = malnutrition 1 = normal nutrition 3 = excess nutrition (overweight, obesity)
X7	Health care education	0 = none 1 = yes
X8	Smoke	0 = never smoke 1 = smoke ago, no smoking now 2 = smoke
X9	Drink	0 = never drink 1 = drink ago, no drinking now 2 = drink

Note: The underlined item represented the dummy variable control.

Table 2. — *Logistic regression analysis of factors that influence newborn health level.*

Variable	β	SE	Wald	*p*	OR	OR 95%CI
X_1	2.541	0.282	92.163	0.000	13.720	1.378 ~ 2.621
X_6	3.217	0.361	123.908	0.000	17.106	4.023 ~ 6.267
X_7	2.724	0.291	101.324	0.000	14.430	2.613 ~ 3.014
Constant	– 3.689	0.346	125.578	0.000	0.082	

Table 3. — *Relationship of hemoglobin level and birth quality in 300 cases of pregnant women.*

Group	Case	Hemoglobin level ($\bar{x} \pm s$, g/l)	F	*p*
< 2,500 g	18	106.19 ± 9.04▲	18.93	0.000
2,500 ~ 3,000 g	128	108.75 ± 12.82*		
3,000 ~ 3,500 g	132	113.32 ± 12.65•		
> 3,500 g	24	116.21 ± 8.74		

Note: ▲ vs 3000 ~ 3500 g, t = 5.61, $p < 0.05$, vs > 3500 g, t = 6.25, $p < 0.05$; * vs 3,000 ~ 3,500 g, t = 5.18, $p < 0.05$, vs > 3,500 g, t = 8.02, $p < 0.05$; • vs > 3,500 g, t = 4.01, $p < 0.05$

Health education contents

In case of each pregnancy test, pregnancy health education was carried out for gravidae by means of direct and simple language. In the interim, a good doctor-patient relationship was established, and health education advisory services were provided. Pregnancy health education mainly aimed at various factors of influencing birth quality. In addition, positive and effective preventive measures were taken to reduce gravidae complications.

Quality control

According to the purpose and significance of this survey and characteristics of birth quality influence factors of newborns [4], the questionnaire was scientifically designed. At the same time, it was necessary to pay attention to the means, quality, and confidentiality of the questionnaire survey.

Statistical analysis

SPSS18.0 software package was used to conduct descriptive analysis for data, including normality test, t test, analysis of variance, and logistic regression analysis. In addition, one-way ANOVA was used for comparison among multiple groups, and LSD-t test was used for comparison between two groups (α = 0.05 as significance level).

Results

Logistic regression analysis result of birth quality influence factors of newborns

For analyzing the correlations of several factors with birth quality of newborns, the analyzed factors were first quantified, and specific quantification scheme is shown in Table 1. After various analysis factors were included into logistic regression equation, the results showed that birth quality influence factors of newborns were: age, nutrition situation, and pregnancy healthcare education of gravidae and puerpera. Specific data are shown in Table 2.

Correlation of birth quality of newborns with hemoglobin level in gravidae body

According to situations of birth weight of newborns, gravidae were divided into several groups. Table 3 compares antepartum hemoglobin levels of various groups of gravidae. For the correlation of birth weight of newborns with antepartum hemoglobin level of gravidae, it was obtained that r = 0.746, and this correlation coefficient had a statistical significance ($p < 0.05$).

Discussion

Birth quality of newborns is one of the key factors of affecting population quality, and it has become an increasingly main indicator of measuring the healthcare development level of newborns in a region [1]. There are a variety of factors influencing birth quality of newborns, including: society, economy, environment, population, and other factors [13-18]. For possible influencing factors of birth quality of newborns analyzed in this study, after logistic regression analysis, it was found that in these factors, influence factors included: age, nutrition situation, and pregnancy healthcare education of gravidae and puerperal, suggesting that the latter factors was greatly related to birth quality of newborns.

Some studies [5] found that for gravidae with hemoglobin increase, the incidence rate of low birth weight newborn apparently increased. Some gravidae in pregnancy stage have no adequate blood volume increase, which causes pachemia and hemoglobin increase and thus causes gestational hypertension and antepartum eclampsia and influences nutrient exchange between placenta and fetus. Therefore, fetal growth and development are restricted [19-21]. In this survey, it was found that hemoglobin level of gravidae in the group with birth weight less than 2,500 g was significantly less than that of the group with birth weight from 3,000 to 3500g and the group with birth weight over 3,500g. Hemoglobin level of gravidae in the group with birth weight from 2,500 to 3,000 g was significantly less than that of the group with birth weight from 3,000 to 3,500 g and the group with birth weight over 3,500 g. Also, hemoglobin level of gravidae in the group with birth weight from 3,000 to 3,500 g was significantly more than that of the group with birth weight over 3,500 g. Correlation analysis showed that birth weight of newborns was positively related to antepartum hemoglobin level of gravidae ($r = 0.746$, $p < 0.01$). It is suggested that birth weight of newborns and antepartum hemoglobin level of gravidae have a certain correlation, indicating that decrease of hemoglobin level of gravidae will increase the risk of occurrence of low birth weight newborns.

Conclusion

This study showed that strengthening health monitoring of gravidae and conducting health education, can reduce incidence rate of maternal and fetal complications and effectively enhance health situations of newborns.

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The role of hysteroscopy in the diagnostic work-up of infertile asymptomatic patients

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Summary

Purpose of investigation: To demonstrate that office hysteroscopy has a key-role in the diagnostic work-up of infertile couples. *Materials and Methods:* The entire database of hysteroscopies performed in 572 menstruated women from 2008 to 2011, was retrospectively analyzed. A two-dimensional correspondence analysis among endometrial patterns, age ranges, and indication for hysteroscopies was made. A main-effect hierarchical log-linear model was built to assess the goodness of the correspondences found. *Results:* A clear cluster of aggregation appears in case of both primary and secondary infertility, with and without other indications for hysteroscopy, as well as in case of primary infertility with irregular menstrual bleeding. In such patients, chronic endometritis, normal pattern, and uterine malformations were frequently found. The most significant correspondence was found for normal pattern and chronic endometritis in case of secondary infertility and primary infertility, respectively. *Conclusions:* Office hysteroscopy should be reconsidered in the diagnostic work-up of infertile couples. It is able to assess or rule out endometrial factor for female infertility.

Key words: Hysteroscopy; Infertility; Asymptomatic women.

Introduction

Infertility affects about 15% of couples worldwide [1,2]. The overall incidence of infertility has remained stable over the past decades [2]. Treatment options and success rates vary with the cause of infertility [3]. Infertile couples are usually advised to begin their investigations after 12 months of attempting to conceive or after six months if the female partner is more than 35-years- old or immediately if there is an obvious cause for their infertility or subfertility [4]. Since 1995, the preliminary advised investigations for the infertile couple have focused on semen analysis, detection of ovarian function by hormonal assay, and evaluation of tubal patency by hysterosalpingography or laparoscopy [5]. Currently there are no relevant differences that have been published concerning the guidelines for the basic evaluation of infertile couples. In fact, although hysteroscopy is the gold standard procedure for uterine cavity exploration, guidelines [6] recommend hysterosalpingography alone in the diagnostic work-up of infertile women. Hysteroscopy is only recommended when clinical or complementary exams (ultrasound, hysterosalpingography) suggest the presence of intrauterine abnormality or after in vitro fertilization (IVF) failure [7]. Moreover the effectiveness of removal intrauterine pathologies to improve the reproductive outcome is still under debate, even if some centres adopt the policy to perform hysteroscopy routinely in infertile patients [6, 8-10].

In 2010, a systematic review of Bosteels *et al.*, reported scarce evidence of the effectiveness of hysteroscopic removal of uterine pathologies (endometrial polyps, sub-mucosal myomas, and intrauterine adhesions) or hysteroscopic metroplasty for improving fertility rate before IVF or intrauterine insemination (IUI) [11]. This review is limited to few randomized and controlled trials, and did not consider the effect of some other pathologies that may be involved in female fertility, such as endometritis, that was found to be associated to a wide number of female fertility problems [12, 13].

Thus the aim of this study was to assess, in a large number of cases, the most frequent findings in both asymptomatic and symptomatic patients that underwent hysteroscopic examination with a diagnosis of infertility in order to better define the role of hysteroscopy in the management of infertile women.

Materials and Methods

Between 2008 and 2011, the hysteroscopic database of the Service of Gynecological Endocrinology and Physiopathology of Reproduction of the Institute of Obstetrics and Gynecology of Foggia, was analyzed. More in detail, the hysteroscopic reports and the relative indications for undergoing hysteroscopy of 572 menstruated patients were analyzed. As a policy of the Institute, patients with sonographic abnormal patterns of endometrial profile and patients with sub-fertility or infertility routinely underwent hysteroscopy, along with the ones with usual indications for hysteroscopy. Moreover the policy of "see and treat" of uterine pathologies in an office setting [14] and endometrial hysteroscopic biopsies were routinely performed. This extensive use of office hysteroscopy provides a better estimation of hysteroscopic findings in women with infertility, and was used to build a model of the likelihood for endometrial pathologies according with indications for hysteroscopy. Therefore, a number of patients analyzed

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(106 out of 572) were symptomatic with or without sonographic abnormalities. Another part (77 out of 572) of patients were asymptomatic and had a normal sonographic examination.

The indications of hysteroscopy were summarized as following: primary infertility (without other indications for hysteroscopy), secondary infertility (without other indications for hysteroscopy), primary infertility with irregular menstrual bleeding, secondary infertility with irregular menstrual bleeding, primary infertility with other indications, secondary infertility with other indications, irregular menstrual bleeding (without infertility), and other indications (without infertility) (Table 1). Under "other indications" the following were grouped: abnormal sonographic endometrial patterns, presence of a cervical polyp, pap smear abnormalities needing to be addressed with an endocervical evaluation, and in case of vaginal polyp. Endometrial pattern retrieved were: normal pattern, endometrial polyp(s), cervical polyp(s), sub-mucosal myoma, chronic endometritis, cervicitis, uterine malformation(s), dysfunctional endometrium, malignancies, synechiae, vaginal polyp, and tubal micropolyps.

In order to check factors involving infertility that may condition endometrial patterns, the patients' age, and the polycystic ovary syndrome (PCOS) have been considered in the likelihood model. Unfortunately, no other causes of infertility were reported in the database, so no other causes of infertility were considered in the model.

Two-dimensional correspondence analysis was used to provide a perceptual map of the correspondence among indications for hysteroscopy and hysteroscopic findings, patients' age,and PCOS. The less far is the distance among the points in the map (hysteroscopy indications, PCOS, patients' age, and patterns points), the stronger was the correspondence found.

To control if the correspondence was significant, the main effect hierarchical log-linear model was built, assessing the behavior of standardized residuals. The behavior of standardized residuals indicated which were the more significant correspondences.

Table 1. — *Descriptive statistic: rates.*

Indications for hysteroscopy	
Primary infertility	57 (10%)
Secondary infertility	20 (3.5%)
Primary infertility with irregular bleeding	13 (2.3%)
Secondary infertility with irregular bleeding	7 (1.2%)
Primary infertility with other indications	27 (11.4%)
Secondary infertility with other indications	11 (1.9%)
Irregular bleeding	106 (18.5%)
Other indications	375 (65.6%)
Hysteroscopic findings	
Normal pattern	141 (24.7%)
Chronic endometritis	68 (11.9%)
Cervicitis	19 (3.3%)
Endometrial polyps	205 (55.1%)
Cervical polyps	86 (15%)
Sub-mucosal myoma	58 (10.1%)
Malignancies	6 (1%)
Uterine malformations	35 (6.1%)
Synechiae	9 (1.6%)
Tubal micropolyps	1 (0.2%)
Vaginal polyp	1 (0.2%)
Dysfunctional endometrial patterns	82 (14.3%)
PCOS	7 (1.2%)
< 21 years old	8 (1.4%)
21 – 30 years old	81 (14.2%)
31 – 40 years old	279 (48.8%)
41 – 50 years old	170 (29.7%)
> 50 years old	34 (5.9%)

Results

Table 1 describes the rate of indications for hysteroscopy and hysteroscopic findings among the 572 patients analyzed. Additionally, Table 1 describes the rate of PCOS cases and the rate of the patients' class age. Among 375 cases grouped as "other indications", 286 (76.3%) patients were addressed to hysteroscopy for abnormal sonographic scan examination of endometrial pattern, 86 (22.9%) for cervical polyps, one for a vaginal polyp (0.3%), and two for abnormal pap smear (0.5%). Dysfunctional endometrial patterns were described as "atrophic endometrium" (five cases – 6.1%), "focal cystic atrophic pattern" (two cases – 2.4%), "hypotrophic endometrium" (one case – 1.2%), "hyperplastic endometrium" (41 cases – 50%), "hypertrophic endometrium" (two cases – 2.4%), "secretive thickened endometrium" (nine cases – 11%), "endometrium not appropriate for the cycle phase" (25 cases – 30.5%), "decidualized endometrium" (one case – 1.2%).

Results from two-dimensional correspondence analysis are depicted in Figure 1. The squared block points in Figure 1 identified the indications for hysteroscopy, while the circular points are the patterns that grouped together patients' age and PCOS. Three clusters of aggregations could have been identified. The first one encompasses patients under 21 and over 50 years of age, undergoing hysteroscopy for other indications than infertility. The hysteroscopic findings in those patients were: vaginal polyp, sub-mucosal myoma, endometrial malignancies, endometrial polyp, and cervical polyp (Figure 1). The second cluster of aggregation encompasses patterns of cervicitis or dysfunctional endometrium, observed in patients with irregular bleeding, aging usually between 31 and 40 years. The third cluster of aggregation appears in cases with both primary and secondary infertility, with and without other indications for hysteroscopy, and in primary infertility with irregular menstrual bleeding. In these patients, usually aging between 21 and 30 years, chronic endometritis, normal hysteroscopic pattern, and uterine malformations were more often found. Patients undergoing hysteroscopy for secondary infertility with irregular bleeding do not seem to have a likelihood of more common hysteroscopic findings. Moreover, PCOS and synechiae points are far from points of the indications for hysteroscopy, suggesting poor correspondence with specific indications for hysteroscopy. The pattern of tubal micropolyps is the most far from anything, suggesting no correspondence.

The main-effect hierarchical log-linear model proves that the overall correspondence found is significant (likelihood ratio: $p < 0.001$; Pearson chi square: $p < 0.001$). Figure 2

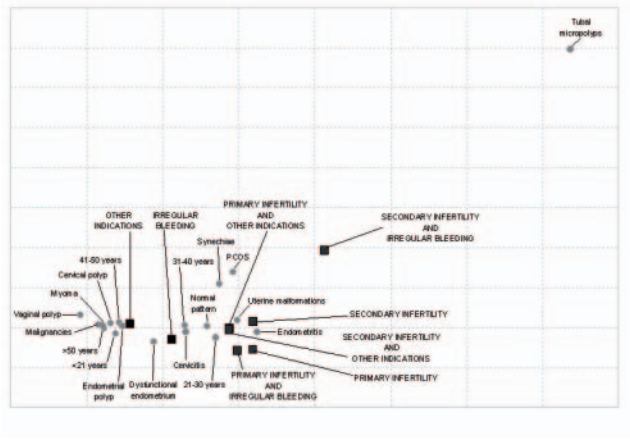

Figure 1. — Two-dimensional correspondence analysis.

depicts the behavior of the standardized residuals, highlighting where the correspondence was more significant. To facilitate the interpretation, the value of the 0,1° percentile on the left of x-axis and the value of 99,9° percentile on the right of x-axis are highlighted in Figure 2 (indicated by arrows). Bars crossing those limits provide a measure of the best correspondence. No correspondence was found for tubal micropolyps pattern (Figure 2). On the other hand, dysfunctional endometrium was more likely to be found in case of hysteroscopy for irregular menstrual bleeding. PCOS patients do not have specific indication for undergoing hysteroscopy. The PCOS patients are more likely to complain of primary infertility with irregular bleeding and secondary infertility with irregular bleeding (Figures 1 and 2) Normal pattern and chronic endometritis were likely to be found in patients with secondary infertility and primary infertility, respectively (Figure 1 and 2). Less strong correspondence may be found for less extreme percentiles.

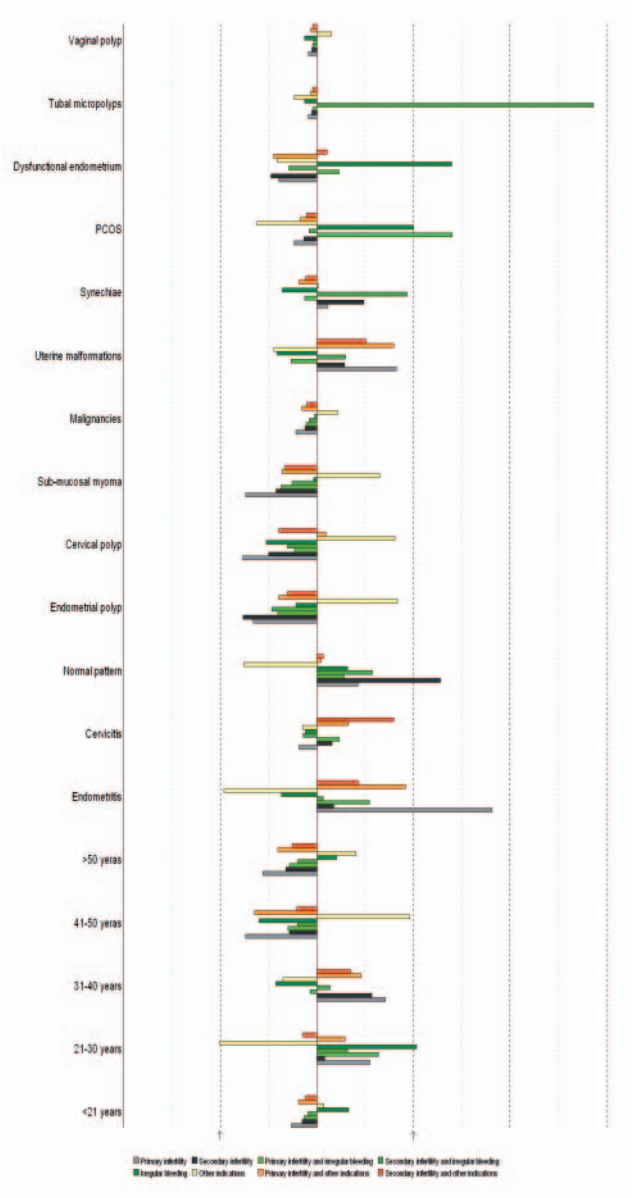

Figure 2. — Behaviour of standardized residuals.

Discussion

According to the aim of the study, the correspondence analysis performed on 572 hysteroscopies, revealed an interesting association of some hysteroscopic findings and the indications to the exam. More in detail, all cases of infertility were found to be more often associated with chronic endometritis (Figures 1 and 2), with the exception of the cases with secondary infertility and irregular menstrual bleeding. However, the limited number of these cases (just seven cases) assessed in this study, biased the absence of association found. It has been reported in literature that chronic endometritis is easily detected by hysteroscopists [13] and that hysteroscopy is the best diagnostic tool for detecting chronic endometritis [15]. The present study highlights that chronic endometritis is often diagnosed at hysteroscopy in infertile asymptomatic patients, suggesting the opportunity to perform hysteroscopy since chronic endometritis is often asymptomatic in such patients. The present findings contradict a recent paper of Kasius *et al.* showing that the clinical implication of CE seems minimal since it can be rarely diagnosed in a population of asymptomatic infertile patients with a normal transvaginal ultrasound examination [16]. On this basis, the present authors can suggest that among infertile female population, hysteroscopy should be indicated also in cases with asymptomatic infertile women without other specific indications to perform hysteroscopy.

A normal endometrial pattern was found most of all in case of secondary infertility which could be in any case, a useful information, in order to exclude an endometrial factor as a possible cause of infertility. It would be interesting to know the associations, if any, between hysteroscopic in-

dications and all the specific causes of infertility or sub-fertility (i.e. premature ovarian failure, endometriosis, and pelvic inflammatory disease), after localizing their points in the Figure 1. This information allows to address the more common cause of female infertility to specific hysteroscopic indications and the relative hysteroscopic pattern. Another interesting finding of the present study is that patients with PCOS did not show any specific indication for hysteroscopy, since the distance of the PCOS point from the squared points of infertility did not differ from the distance from the squared point of the irregular bleeding indications (Figure 1). This leads to speculate that infertile patients with PCOS may show an endometrial dysfunctional pattern which may concur to explain their infertility disorder [17, 18], as well as women undergoing hysteroscopy for irregular menstrual bleeding.

Conclusion

This study demonstrates that chronic endometritis is the most frequent hysteroscopic finding associated to infertility, even in asymptomatic women.

Moreover results showed that hysteroscopy is a key-examination in infertile patients, which is able to detect chronic endometritis in both asymptomatic and symptomatic women. On the other hand, hysteroscopy allows to rule out an endometrial factor for female infertility in the other cases. Thus, in the authors' opinion, the role of office hysteroscopy in the diagnostic work-up of infertility should be reconsidered.

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Clinical analysis of diagnosis and treatment of 13 cases with cesarean scar pregnancy

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Summary

Objective: To explore effective methods in diagnosing and treating cesarean scar pregnancy (CSP) after cesarean section. *Materials and Methods:* The clinical data of 13 cases with CSP who were admitted to the present hospital from October 2009 to February 2012 were retrospectively analyzed . *Results:* The agreement diagnostic rate was 92.3% (12/13). On the basis of transvaginal color Doppler ultrasonography 12 patients had medical therapy combined with uterine artery embolization (UAE) and curettage was successfully performed. One patient was diagnosed through an emergency setting due to symptomatology. *Conclusion:* Early accurate diagnosis of CSP is the key to perform proper and successful treatment.

Key words: Cesarean; Cesarean scar pregnancy (CSP); Ultrasonography; Diagnosis; Treatment.

Introduction

Cesarean scar pregnancy (CSP) is a special type of ectopic pregnancy, and is one of the long-term complication [1]. In recent years the cesarean section rate is on the rise and CSP rate shows an increasing tendency and there was no specificity in clinical situation, maybe as a result of uterine massive hemorrhage or even uterine rupture that threatens the patient's life. Thirteen cases of CSP were reviewed and analyzed in this article.

Materials and Methods

Clinical data of the case

Thirteen clinical cases of CSP in Southeast University Affiliated Jiangyin Hospital were collected from October 2009 to February 2012. Patients' age ranged from 22 to 37 years, with an average of 30 years, and most had a history of multiple pregnancies or cesarean sections. There were nine cases that had menolipsis history, with a time range of 37 to 90 days; nine cases had vaginal bleeding, three cases of bleeding after abortion, and one case with abdominal pain.

Equipment and methods

A transvaginal color Doppler sonography with type C8-4v probe and 5.5~7.0 MHz frequency was used. Observation contents included incision lesions' morphology, size, internal echo, and blood supply of anterior wall of uterus bottom. Dynamic observation with ultrasonography was performed every week after conservative treatment. A chemiluminescence immunoassay was used to assess blood β-hCG values at one day before conservative treatment, at three days and seven days after treatment, and then every two weeks until the β-hCG levels decreased to normal value (< 2.9 mIU/ml).

Diagnostic criteria

Patients' clinical manifestation and urine pregnant tests or β-hCG confirmed the pregnancy and CSP was diagnosed through ultrasonography. Godin first reported that vaginal ultrasonography's imaging characteristics of cesarean scar pregnancy in 1997 [2], and formulated strict diagnostic criteria: 1) no gestation sac in uterus; 2) no gestation sac in cervical canal; 3) gestation sac grow in isthmus of anterior wall of uterus; 4) uterine wall among bladder and gestation sac is week.

Treatment methods - drug treatment

Conservative drug treatment is suitable for the cases without symptoms and with hemodynamic stability, uterus did not rupture, and pregnancy time < 8 weeks, thickness of muscular layer between CSP and bladder < 2mm [3]. Methotrexate (MTX) and mifepristone were used for treatment. The dosage of MTX was a single-dose muscle injection of 50mg/m^2 and mifepristone followed with a dose of 50 mg each time, twice daily, orally, for five days. The administration of MTX is adjusted according to any eventual β-hCG fluctuations. The patients were closely observed for any serious side-effects as bone marrow suppression, injury of liver and renal function, and so on during the drug treatment; it was stopped if they appeared.

Uterine artery embolization (UAE): embolization through femoral artery is performed and can be reached from one or from both sides for an emergency hemostasis of patients in critical condition and 100 mg of MTX was introduced in the vessels.

Surgical treatment

Dilatation and curettage: once CSP is diagnosed, dilatation and curettage is not recommended immediately, as it may lead to massive vaginal bleeding, perforation of uterus, etc. Dilatation and curettage was performed under ultrasound after drug treatment or UAE.

Trans-abdominal operation: Local lesion resection and uterus neoplasty or dilatation and curettage included. Local lesion resection is suitable for conservative drug treatment or those not effective after UAE; dilatation and curettage is only suitable for vaginal bleeding that cannot be controlled by conservative treatment or CSP patients with no reproductive function requirement.

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Table 1. — *Clinical feature and basic information of 13 CSP cases.*

Case	Age	Menolipsis history	Vaginal bleeding history	Abdominal pain history	Pregnancy history	Cesarean times	Time from last cesarian
Cesarian (yrs)							
1	27	none	22 days	none	3 pregnancies, 1 birth	1	7 years
2	32	45	Bleeding after drug abortion	none	2 pregnancies, 1 birth	1	9 years
3	26	43	Half a day	none	4 pregnancies, 2 birth	2	1.5 years
4	34	12 weeks	Bleeding 2 months after abortion	none	5 pregnancies, 2 birth	2	0.5 years
5	35	none	40 days	none	10 pregnancies, 2 birth	2	5 years
6	29	39	1 day	none	1 pregnancies, 1 birth	1	8 years
7	27	37	3 days	positive	4 pregnancies, 1 birth	2	3.5 years
8	26	lactation period	2 months	none	2 pregnancies, 1 birth	1	9 months
9	22	55	half a month	none	3 pregnancies, 1 birth	1	3.5 years
10	28	38	6 days	none	4 pregnancies, 1 birth	1	4 years
11	31	41	1week	none	4 pregnancies, 2 birth	2	2 years
12	31	55	none	none	2 pregnancies, 1 birth	1	2.5 years
13	37	unrevealed	Bleeding after drug abortion	none	4 pregnancies, 2 birth	1	5.5 years

Table 2. — *Lab tests, ultrasound performance and treatment in 13 CSP cases.*

Eg.	Lab. test		Ultrasound test			Treatment	Discharge diagnosis
	HGB (g/l)	Blood β-hCG (mIU/ml)	Diagnosis	Lesion size	Blood		
1	110	6,748	twice	4.0×2.7cm	rich	drug	CSP
2	125	11,109	twice	3.1×1.6cm	rich	drug	early pregnancy + CSP
3	123	96.96	once	1.2×1.0cm	rare	drug	CSP
4	46	5.47	twice	3.1×1.8cm	rich	drug + UAE	CSP + hemorrhagic shock
5	96	4,870	twice	3.1×2.6cm	rich	drug + UAE	CSP + anemia
6	113	28,679	once	1.6×1.1cm	rich	drug + D&C	CSP
7	106	39,385	once	3.8×1.3cm	relatively rich	drug+ D&C	CSP + anemia
8	111	2,987	twice	3.3×2.6cm	rich	drug + D&C	CSP
9	119	1,579	once	1.3×0.8cm	rich	drug + UAE + D&C	CSP
10	123	26,940	once	2.2×1.0cm	rich	drug + UAE + D&C	CSP
11	105	123,867	twice	5.4×5.3cm	rich	drug + UAE + D&C	CSP + anemia
12	126	26,984	once	1.9×1.2cm	rich	drug + UAE + D&C	CSP
13	55	7,504	misdiagnosis	8.1×5.4cm	rich	Laparotomy: local lesion resection + subtotal hysterectomy	CSP+ hemorrhagic shock

Notions: drug: MTX with mifepristone; UAE: uterine arterial embolization

Efficacy evaluation

Clinical symptoms and physical sign disappear after treatment, blood β-hCG value reduce to common value (< 2.9 mIU/ml), incision's scar lesion at uterus bottom disappear under ultrasound show be healed.

Results

Thirteen CSP cases had β-hCG test before treatment and the values ranged from 5.47 to 123,867 mIU/ml. Twelve out of 13 (92.3%) cases were diagnosed once or twice by transvaginal color Doppler sonography (typical ultrasonogram as Figure 1), of which six cases were diagnosed at first time (46.2%, 6/13), and other six cases were diagnosed at second time, among which one case of early pregnancy was complicated with CSP. One case was transferred form another hospital where an ultrasonography was performed due to constant vaginal bleeding after early pregnancy abortion. A rich blood ventricosity mass of 8.1 × 5.4 cm was detected and a local lesion resection and subtotal hysterectomy was performed, and pathology verified CSP after operation. Clinical manifestation, pregnant production history, lab test related, ultrasonography and treatment of this group are shown in Table 2. Drug side-effects as bone marrow suppression, injury of liver, and altered renal function were not reported. Follow-up visit continued 1.5 to six months after leaving the hospital, β-hCG values reduced to normal levels, and incision's scar lesion at uterus bottom disappeared under ultrasound.

Discussion

The incidence of CSP after cesarean occurs in 0.45% and 6.1% in ectopic pregnancy that have cesarean history

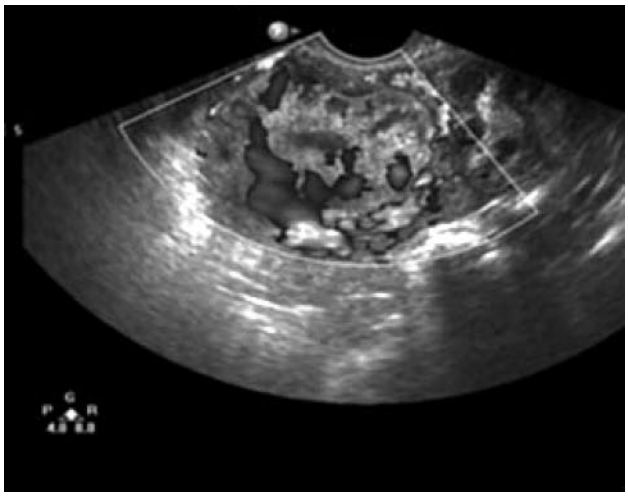

Figure 1. — Typical CSP transvaginal color Doppler sonography, with rich flow signal seen in mixed mass.

[4]. Etiology of CSP is still unknown and it may be related to poor healing of incision after cesarean as suggested by Jiaoguangyu *et al.* [5], it may be related with rapid fertilized egg movement or fertilized ovum growth retardation resulting in fertilized ovum implantation on uterus scar. Transvaginal color Doppler sonography is an important method for the diagnosis of CSP. Color Doppler imaging especially has an important value to observe the blood flow situation of lesions; 86% of this group show rich flow signal around lesion (Figure 1). In case of CSP, the trephocytes of the placenta villi intrude into uterus scar, destroy local blood vessels, and for this reason during uterine contraction may result a sudden hemorrhage that can be life-threatening if not treated correctly or on time; three cases of this group had conspicuous vaginal bleeding (1,000~2,000 ml) after uterine curettage. The misdiagnosis rate is high for the low rate of CSP and sonographer is not familiar with CSP: in this group six cases (6/13) were diagnosed as CSP at first ultrasound, six cases misdiagnosed were treated with misoprostol for abortion, and diagnosed as CSP at second ultrasonic testing for persistent vaginal bleeding, two cases had misoprostol abortion combined with CSP. In another case that was transferred into the present hospital because of massive vaginal bleeding after drug-induced abortion, and dilatation and curettage of the uterus in other hospital, ultrasound showed rich blood mass diagnosed as CSP after laparotomy operation performed due to the critical situation of the patient.

There is no uniform clinical treatment at present for CSP, to reduce bleeding, preserve reproductive function, and survival. The aim of MTX treatment, which is a folic acid antagonist drug that persists as thymidine phosphorylase and purine nucleotide intracellular 24 hours after administration, destroying trophoblast and causing rapid fetal death, with definite effect, few side-effects, and does not increase pregnancy abortion rate in the following pregnancies [6]. Mifepristone is a type of progesterone receptor antagonist that can necrotize the membrane organization, and determine the death of the fetus. MTX combined with Mifepristone is a safe and reliable method of conservative medication at present [7]. The proposed UAE is a type of treatment that can avoid blood transfusion, reach hemostasis which is needed to preserve the uterus, while inducing an abortion [8]. In six cases CSP was diagnosed first by ultrasound, β-hCG value was 96.96 ~ 39,385 mIU/ml, lesion maximum diameter 1.2 ~ 3.8 cm, with five out of six case of cases < three cm. Two of these cases were treated with conservative therapy; other two cases were treated with conservative treatment for one week. Ultrasound showed blood supply to be reduced and dilatation and curettage of the uterus was performed under ultrasound guidance, and intraoperative blood loss was < 50ml. The other two cases were treated with a combination of medication, by UAE, and by dilatation and curettage of the uterus. The six cases of CSP were diagnosed by a second ultrasound and β-hCG value was 5.47 ~ 123,867 mIU/ml, and the size of the lesion was 3.1~ 5.4 cm. Doppler ultrasound showed rich blood flow signals ring in lesion, one case of medication, two cases combination of medication and UAE, two cases combination of medication and dilatation and curettage of the uterus, one case combination of medication, UAE and dilatation and curettage of the uterus: all cases healed. One patient was transferred into the present hospital because of massive vaginal bleeding in the progress of dilatation and curettage of the uterus in other hospital; ultrasound showed ventricosity mass in uterus bottom, Hb 55 g/l, BP: 72/45 mmHg, performed rescue treatment including blood transfusion, uterine packing, etc. immediately, and performed emergency laparotomy. Uterus' shape resembled a calabash during operation, with a bottom expansion of approximately 7 × 8 cm. Blood vessel was rich with hyacinthine-like surface, serosal layer was intact, under great tension, and preserving the uterus was difficult, therefore a subtotal hysterectomy was performed. Uterine form was regular after open resection, dark red necrosis tissue was seen in bottom, chorionic villi was proved with pathology.

Through a review of 13 cases treated for CSP the authors can state conclude: 1) ultrasound is an important tool for diagnosis of CSP and has an important value in the monitoring of lesion size and blood supply, and in choosing the right treatment; 2) make sense of CSP when faced with cesarean patients that have massive vaginal bleeding or bleeding continuously after abortion, in cases where treatment cannot be blindly continued, and checked with ultrasound by experienced sonographer for diagnosis and next treatment; 3) for those in early-stage CSP with small lesion inn which villus invades slightly into muscular layer, drug treatment that causes the death of the embryos in one wee, and performing dilatation and curettage has a positive ef-

fect; 4) for case with large lesions with rich blood supply and massive vaginal bleeding, treatment of UAE combined with MTX injection has a good effect, and dilatation and curettage can be performed one week later.

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A deceiving disease in women for clinicians: peritoneal tuberculosis

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Summary

Introduction: Peritoneal tuberculosis (TB) is uncommon in developed countries, although there is an increase in incidence due to the patients with acquired immunodeficiency syndrome and in immigrants from countries with tuberculosis. The aim of the study was to identify characteristic features of peritoneal tuberculosis (TB), which may be useful for the clinical differential diagnosis and management of this deceiving disease. *Materials and Methods:* For this retrospective study, 18 patients, who were diagnosed with peritoneal TB were identified after surgery. *Results:* Initial presentation consisted of ascites, pelvic masses, and elevated levels of CA-125. All patients were initially misdiagnosed as ovarian carcinoma. Tissue biopsies obtained from laparoscopy or laparotomy revealed accurate diagnosis of peritoneal TB. *Conclusion:* Peritoneal TB should be included in the differential diagnosis of ascites and pelvic masses and can be accurately diagnosed by laparoscopic biopsy.

Key words: CA-125; Ovarian carcinoma; Peritoneal tuberculosis.

Introduction

Improved nutrition, effective vaccination programs in endemic countries, and wide usage of chemotherapy decreased the incidence of tuberculosis (TB) during the second half of the 20th century especially in developed countries as well as developing countries. However, by the end of the century due to the spread of human immunodeficiency virus (HIV) infection and increased *Mycobacterium tuberculosis* resistance to antituberculous agents [1], there has been resurgence.

Although peritoneal TB is uncommon in the developed countries, its incidence has increased in the developing countries especially among acquired immunodeficiency syndrome (AIDS) patients. Subsequently, in the Western world, peritoneal TB is usually found in AIDS patients and foreign-born individuals [2-5]. The World Health Organization (WHO) reported an estimated 9.4 million cases of TB globally in 2009, while 7% of the cases were from Mediterranean countries [6]. Peritoneal TB is estimated to account for 1-2% of all TB cases and its association with pulmonary disease is not well defined [7]. The exact diagnosis can only be confirmed by tissue biopsies obtained from laparoscopy or laparotomy.

Clinical manifestations of peritoneal TB may resemble those of ovarian carcinoma with ascites, pelvic masses, and elevated levels of CA-125. A clinician who is not well aware of peritoneal TB may be deceived by the presentation and sometimes even by the operative findings. Therefore, patients with peritoneal TB may be misdiagnosed as having ovarian carcinoma and subjected to unnecessary extended treatment and surgery.

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Turkey is an east Mediterranean country that receives immigrants especially from the Middle East as well as some of the former Soviet republics. In this paper, the authors present 18 cases of peritoneal TB in a developing country setting. The aim of the study was to identify characteristic features of peritoneal TB, which may be useful for the clinical differential diagnosis and management of this deceiving disease.

Materials and Methods

For this retrospective study, 18 patients, who were diagnosed with peritoneal TB in the gynecologic oncology clinic of Istanbul University School of Medicine, were identified. The study protocol was approved by the Ethics Committee of Istanbul University School of Medicine and informed consent was waived due to the retrospective nature of this study. The diagnosis of TB was confirmed, if pathology results revealed caseification necrosis and granulomatous inflammatory reaction and if culture results were positive for TB. The samples were obtained by biopsy from the inflammatory peritoneal tissue during either laparoscopy or laparotomy.

Thorough physical examination, complete blood count, routine biochemical tests, tumor markers such as CA-125 levels, chest x-ray, ultrasonography, and magnetic resonance imaging (MRI) were performed in all cases.

Results

Patient overview is presented in Table 1. The mean age of the patients was 53 years (range 18 to 75). Eight of the 18 patients were in the postmenopausal period. Most common symptoms were irregular vaginal bleeding, abdominal pain, ascites, weight loss, and fatigue. None of the patients had a family or past history for TB. One patient had breast cancer. Chest X-ray did not show any evidence acute or

Table 1. — *Overview of 18 patients with peritoneal TB.*

Case No.	Age	Ascites	Pre-operative CA-125	Ultrasonography and MRI findings	Preliminary diagnosis	Operation	Frozen section for TB	Complications
1.	28	(+)	127	Semisolid mass	Ovarian carcinoma	LPT; multiple bx	(+)	(–)
2.	53	(+)	361	Bilateral semisolid mass	Ovarian carcinoma	LTP; TAH+BSO+P.Omm+ Pelvic/para-aortic LA	(–)	(+) wound infection
3.	58	(–)	23	Pelvic solid mass	Ovarian carcinoma	LPT; multiple bx	(+)	(–)
4.	70	(+)	95	Pelvic solid mass and peritoneal thickening	Ovarian carcinoma	LPT; multiple bx	(+)	(+) bowel injury
5.	55	(+)	77	Multicystic pelvic mass	Ovarian carcinoma	LPT; multiple bx	(+)	(–)
6.	40	(+)	106	Unilateral multicystic mass	Ovarian carcinoma	LPT; TAH+salpingectomy	(+)	(–)
7.	55	(+)	431	Bilateral semisolid mass	Ovarian carcinoma	LPT; TAH+BSO+P.Omm.+ Pelvic/para-aortic LA	(–)	(–)
8.	42	(+)	245	Multicystic pelvic mass with solid component	Ovarian carcinoma	LPT; TAH+USO	(–)	(–)
9.	49	(+)	178	Bilateral solid ovarian tumors (Breast cancer patient)	Metastatic ovarian carcinoma	LTP; TAH+BSO	(+)	(+) wound infection
10.	75	(+)	472	Peritoneal thickness	Ovarian carcinoma	LTP; TAH+BSO+P.Omm.	(–)	(–)
11.	47	(+)	142	Bilateral semisolid mass	Ovarian carcinoma	LTP; TAH+BSO	(–)	(–)
12.	30	(+)	87	Bilateral solid ovarian mass	Ovarian carcinoma	L/S; multiple bx	(+)	(–)
13.	50	(+)	129	Peritoneal thickening, omental cake	Ovarian carcinoma	L/S; multiple bx	(+)	(–)
14.	66	(–)	233	Unilateral semisolid ovarian mass	Ovarian carcinoma	L/S; USO	(+)	(–)
15.	18	(+)	437	Bilateral semisolid ovarian mass	Ovarian carcinoma	L/S; multiple bx	(+)	(–)
16.	43	(–)	112	Unilateral ovarian solid mass	Ovarian carcinoma	L/S; USO	(+)	(–)
17.	23	(–)	109	Unilateral ovarian solid mass	Ovarian carcinoma	L/S; cystectomy (right ovary)	(+)	(–)
18.	38	(+)	212	Unilateral ovarian solid mass	Ovarian carcinoma	L/S; USO	(+)	(–)

BSO: bilateral salpingo-oophorectomy, USO: unilateral salpingo-oophorectomy, Bx: biopsy, LA: lymphadenectomy, LPT: laparotomy, P.Omm.: partial omentectomy, TAH: total abdominal hysterectomy, L/S: laparoscopy.

chronic pulmonary disease in any of the patients. Vital signs were within normal limits for all patients.

The preliminary diagnosis was ovarian carcinoma for all patients (metastatic ovarian carcinoma for the breast cancer patient). Mean of CA-125 level was 198.67 U/ml (range 23 U/ml to 472 U/ml). Four patients had CA-125 levels < 100 U/ml.

After the complete preoperative evaluation, 11 patients (61%) were managed with laparotomy while seven patients (39%) underwent laparoscopy. Seven of the 11 patients in the laparotomy group underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omental biopsy. Three patients underwent third-look laparotomy. One patient underwent unilateral salpingo-oophorectomy. Three of the seven patients in the laparoscopy group underwent unilateral salpingo-oophorectomy and one patient underwent right ovarian cystectomy. Three patients underwent biopsy. Frozen section biopsy was performed in all patients. Frozen section biopsy was negative for malignancy in all patients, while positive for TB in 13 out of 18 patients (72%). The final evaluations of the specimens revealed granulomatous disease consistent with TB and no evidence of malignancy in all patients. No complications were reported in laparoscopy group, while two patients in the laparotomy group had postoperative wound infection and one patient had a bowel injury during surgery. All patients were referred to a phthisiologist postoperatively.

Discussion

The common diagnosis in patients with ascites, pelvic masses, and elevated levels of CA-125 is ovarian carcinoma. In older patients with a family history of cancer, metastatic uterine or fallopian tube malignancies may also result in these clinical findings. Peritoneal TB is significantly uncommon and requires a high index of suspicion.

The symptoms of the patients in the present study were non-specific and non-diagnostic. Most common findings were irregular vaginal bleeding, abdominal pain, ascites, weight loss, and fatigue. In none of the patients the preliminary diagnosis was TB. CA-125 levels were elevated, but the values did not exceed 472 U/ml, differentiating peritoneal TB from advanced stage disseminated ovarian carcinoma where CA-125 levels are usually higher. In line with the present findings, in most previous reported cases of peritoneal TB, CA-125 levels were < 500 U/ml [3-5, 8-9]. On the other hand, there was one recently reported case of peritoneal TB with a highly elevated level of CA-125 (2567.6 U/ml) [10]. Therefore, it is still advisable that patients who present with pelvic masses, ascites, and elevated levels of CA-125 should be preliminarily diagnosed with ovarian carcinoma until proven otherwise. Thus, the use of CA-125 as a differential diagnosis between peritoneal TB and ovarian carcinoma may be inconclusive and sometimes misleading.

The diagnosis of peritoneal TB is best made through evaluation of suspect tissue. Typically, infected tissue demonstrates granulomatous inflammation with central necrosis, which is extremely suggestive of peritoneal TB [7]. Laparoscopy seems to be an efficient and safe method to provide tissue samples because of the lower risk of complications when compared with laparotomy.

Conclusion

The rarity of peritoneal TB contributes to a low index of suspicion and a low incidence of accurate preoperative diagnosis. When peritoneal TB patients with ascites and a pelvic mass are misdiagnosed with ovarian carcinoma, they may undergo unnecessary extensive abdominal and pelvic surgeries. Hence, peritoneal TB should be included in the differential diagnosis of ascites and pelvic masses and can be accurately diagnosed by laparoscopic biopsy.

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Establishment of reference range for thyroid hormones in normal pregnant women in China's coastal area

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Summary

Purpose: The current study aims to establish reference ranges for thyroid hormones in normal pregnant women during their pregnancy period. *Materials and Methods:* A one-time cross-sectional survey was conducted on 490 normal pregnant women and 51 non-pregnant women (control). The serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3), and free tetraiodothyronine (FT4) levels were measured. *Results:* The serum FT3 and FT4 levels in pregnant women decreased gradually from the first to the last three months of pregnancy ($p < 0.01$). The serum TSH level increased gradually during the whole pregnancy ($p < 0.01$), and was significantly lower than the control ($p < 0.01$) in the first three months. However, in the middle and last three months of pregnancy, TSH was higher than the control ($p < 0.01$). *Conclusions:* The thyroid hormone levels in normal pregnant women are different from those in non-pregnant women; significant differences exist among the three stages of pregnancy.

Key words: Pregnancy; Stage of pregnancy; Thyroid hormone; Reference range.

Introduction

Thyroid diseases, such as hypothyroidism and hyperthyroidism, are the most common endocrine diseases in women of childbearing age, with an incidence from one to two percent in pregnant women [1]. In the past ten years, studies on thyroid diseases and pregnancy have developed rapidly. The studies focused on the optimized treatment of pregnant women with thyroid diseases, effects of maternal thyroid diseases, and therapeutic strategies on fetal development and pregnancy outcome. Pregnancy causes great changes in thyroid function; maternal thyroid diseases adversely affects pregnancy and fetal growth [2]. Therefore, the proper management of gestational thyroid diseases, which depends on the accurate measurement of thyroid hormone levels for the establishment of proper therapy strategies, can ensure a normal pregnancy and avoid adverse pregnancy outcomes.

The clinical assessment of thyroid function is more difficult for pregnant women because of their high metabolic state. The physiological changes in pregnancy (increase of plasma volume and thyroid binding globulin amount), relative lack of iodine, and difference of iodine nutritional status in different areas implies that the generally uniform reference ranges of thyroid hormones for non-pregnant women are not suitable for pregnant women. During pregnancy, the free triiodothyronine (FT3) and free tetraiodothyronine (FT4) levels gradually decrease, and the thyroid stimulating hormone (TSH) level gradually increases. Therefore, using the normal serum TSH level in non-pregnant women as reference may misdiagnose normal pregnant women with reduced TSH to have hyperthyroidism. Likewise, pregnant women with subclinical hypothyroidism, of whom the TSH level is mildly elevated but less than the high limit of reference range, may be misdiagnosed as normal, which is not an advisable approach to the management of thyroid diseases during pregnancy.

The current evaluation of thyroid function during pregnancy is performed following the manufacturer-provided reference values for non-pregnant women. Studies on reference ranges for thyroid hormones during pregnancy have been reported; however, these established reference ranges are unreliable because the research subjects were from areas with different iodine nutritional status, the sample size was small, the data were restricted to certain three months in pregnancy, and the statistical method used was inappropriate. Mean ± 2SD is used for calculating reference ranges. This study avoids the methodological defects on reports about reference ranges for thyroid hormones during pregnancy using larger sample size and more precise cross-sectional data during different stages of pregnancy. Moreover, the subjects in this study were from the same area (Quanzhou, China), in which there was no report about established reference ranges for thyroid hormones in pregnancy.

In the current study, a one-time cross-sectional survey was conducted on pregnant women in China's coastal area (Quanzhou, China), with appropriate urinary iodine level (100 g/l to 200 g/l) in the population. This study aims to establish specific reference ranges for thyroid hormones in the first, middle, and last three months of pregnancy. The result would be helpful for objective and accurate evaluation of changes in thyroid function for pregnant women in this area, and correct establishment of prevention and treatment measures to ensure normal pregnancy.

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Materials and Methods

Exactly 490 healthy pregnant women (22- to 36-years-old), who were admitted from October 2010 to December 2011 in the maternal and child health hospitals in Shishi, Dehua, and Anxi (Quanzhou, China), as well as in the Second Affiliated Hospital of Fujian Medical University (Quanzhou, China), were enrolled in this study. There were 122 cases in first three months of pregnancy (four to 12 weeks), 240 cases in middle three months (13 to 27 weeks), and 128 cases in last three months (≥28 weeks). Pregnancy was confirmed via ultrasound scanning and pregnancy test in the first three months. The cases with hyperemesis gravidarum, multiple pregnancy, thyroid diseases and other endocrine diseases before pregnancy, preeclampsia, and administration of drugs affecting thyroid function were excluded. Fifty-one healthy non-pregnant women (25- to 42-years-old) in the same area were selected as controls. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Shishi (Overseas Chinese) Hospital. Written informed consent was also obtained from all participants.

Approximately five ml of fasting venous blood was drawn and centrifuged and the serum was separated and stored at -70°C for future testing. The serum FT3, FT4, TSH, thyroid peroxide enzyme antibody (TPOAb), and human chorionic gonadotropin (hCG) levels were measured. The manufacturer-provided reference ranges for non-pregnant women (manufacturer's value) were attached to each test results. The gestational week of blood sample collection was also recorded.

Inter-laboratory quality control was performed in this study. The thyroid hormone concentrations of each sample were detected. The samples were mixed to obtain the high and low quality control samples (QC) for every 100 samples. The expected and measured values of QC and the manufacturer's values are shown in Table 1. The instant method (Grubbs method) [3] was used for the assessment of inter-laboratory quality control (five batches). According to the quality control international system (SI) value table (n = 5: n2s = 1.67, n3s=1.75), the low and high limits of SI were less than 2s, indicating a controllable range. In addition, the measured values were within the range of the expected values (X ± 2S), suggesting that the random error of this batch of sample was small and the data were reliable.

Table 1. — *Expected values, measured values, and SI values of quality control samples.*

Index		FT3 (pmol/l)	FT4 (pmol/l)	TSH (mIU/l)
n		5	5	5
QC_{high}	Expected value	10.97±2.9	35.54±6.95	40.3±36.8
	Measured value	12.29±0.37	34.87±1.54	36.65±1.69
QC_{low}	Expected value	4.21±2.23	11.06±6.97	1.38±0.87
	Measured value	3.9±0.01	13.5±1.07	1.82±0.06
	CV_{high}	3.08	4.42	4.55
	CV_{low}	0.2	7.8	3.13
SI (QC_{high})	High limit	1.349	1.376	0.79
	Low limit	1.2	1.271	1.613
SI (QC_{low})	High limit	1.0	1.5657	0.8032
	Low limit	1.0	1.1438	1.6413

Nadults)ote: $SI_{high\ limit} = (X_{max} - Mean)/S$, $SI_{low\ limit} = (Mean - X_{min})/S$.

Table 2. — *Reference ranges for thyroid hormones of pregnancy, control, and manufacturers's values (percentile).*

Index	Group	n	2.5th	50th	97.5th	Manufacturers's Values
FT3 (pmol/l)	First 3 months	122	3.75	4.59	7.23	3.5-6.5 (95% confidence interval)
	Middle 3 months	240	3.31	4.06	4.93	
	Last 3 months	128	3.16	3.87	4.48	
	Control	51	3.82	4.66	5.61	
FT4 (pmol/l)	First 3 months	122	12.85	18.07	25.3	11.5-22.7
	Middle 3 months	240	12.03	16.68	20.14	
	Last 3 months	128	11.02	15.89	19.43	
	Control	51	11.72	16.05	20.61	
TSH (mIU/l)	First 3 months	122	0.01	1.08	3.79	0.55-4.78 (2.5^{th} and 97.5^{th} percentile, adults)
	Middle 3 months	240	1.09	2.13	4.17	
	Last 3 months	128	1.08	2.39	5.95	
	Control	51	0.62	1.68	3.74	

Statistical analysis

Statistical analysis was performed using SPSS 16.0. Histogram generation and tests of data normality and difference among groups were performed. The ranges of middle 95% (2.5^{th}, 50^{th}, and 97.5^{th} percentiles) for FT3, FT4, and TSH in the three stages of pregnancy were calculated as references. A correlation analysis on the changes of thyroid hormones and hCG was also performed. The thyroid hormone levels of first, middle, and last three months of pregnancy, as well as the control and manufacturer's value, were compared; the differences between the pregnant and non-pregnant patients were analyzed.

The serum FT3 and FT4 levels were calculated from the corresponding mean values. A normal distribution of FT3 and FT4 was observed in the middle and last three months of pregnancy as well as in the control ($p > 0.05$), with normal distribution for FT4 and positively skewed distribution for FT3 in first three months, respectively ($p < 0.05$). The serum TSH level was also calculated from the median. A positively skewed distribution of TSH was noted in each stages of pregnancy ($p < 0.05$). The histograms of thyroid hormones are shown in Figure 1. The homogeneity of variance test showed a heterogeneity of variance for each group (FFT3 = 11.669, $p < 0.01$; FFT4 = 6.064, $p < 0.01$; FTSH = 19.853, $p < 0.01$). Therefore, the Kruskal-Wallis test was used for compare the overall difference among different groups; the Dunnett's T3 test was used for multiple comparisons. Bilateral $p < 0.05$ was considered as statistically significant.

Results

Reference ranges of thyroid hormones in different stages of pregnancy

The 2.5^{th} and 97.5^{th} percentiles of serum FT3, FT4, and TSH concentrations are shown in Table 2. In the first three months of pregnancy, the low and high limits of TSH were 0.01 mIU/l and 3.79 mIU/l, respectively. These values were the lowest among the three stages of pregnancy; lower than the manufacturer's values. The low limit of TSH was lower and the high limit was almost the same compared with the control. During the middle and last three months of pregnancy, the low limit of TSH was higher than the manufacturer's value and the control, respectively. The high limit of TSH during the middle three months was lower than the manufacturer's value, but higher than the control. However, the high limit of TSH during the last three months was higher than the manufacturer's value and control.

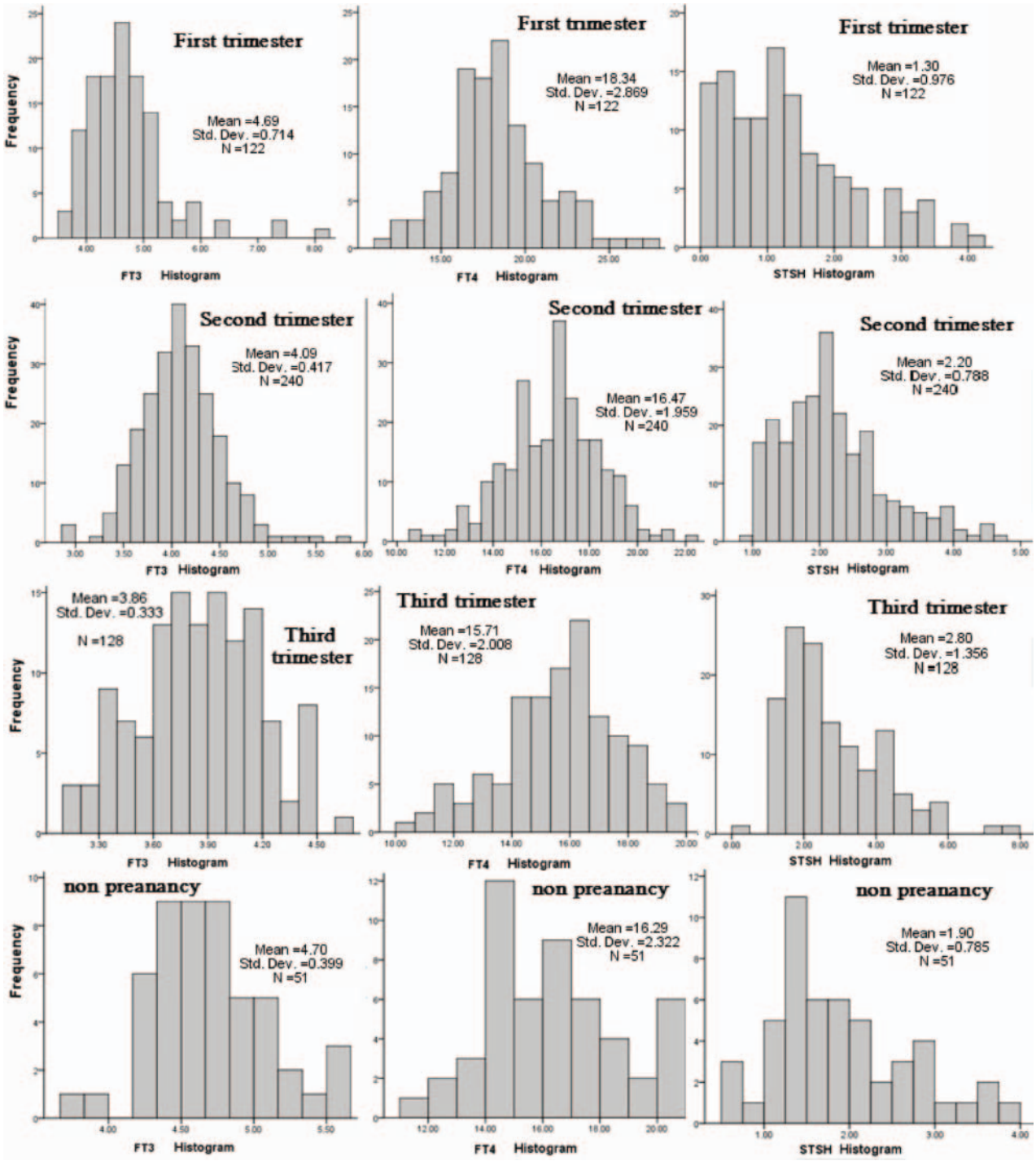

Figure 1. — Histograms of FT3, FT4, and TSH in pregnancy and non-pregnancy.

During the first three months of pregnancy, the low and high limits of FT3 were 3.75 pmol/l and 7.23 pmol/l, respectively, which were both higher than the manufacturer's values. However, the low and high limits during the middle and last three months were lower than the manufacturer's values. In addition, the low limit at each stage of pregnancy was lower than the control. The high limit during the first three months was higher than the control, whereas that during the middle and last three months was lower than the control.

The low and high limits of FT4 during the first three months of pregnancy were higher than the manufacturer's values. The low limit during the middle three months was

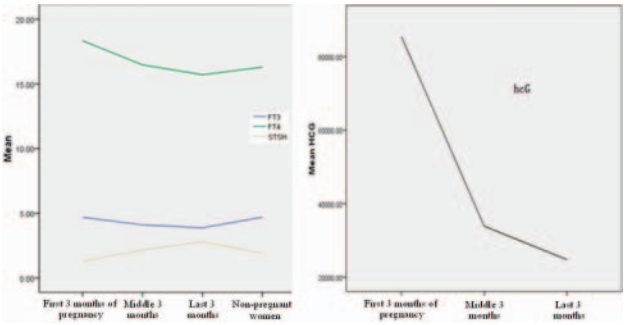

Figure 2. — Variations of thyroid hormones and hCG levels during different stages of pregnancy.

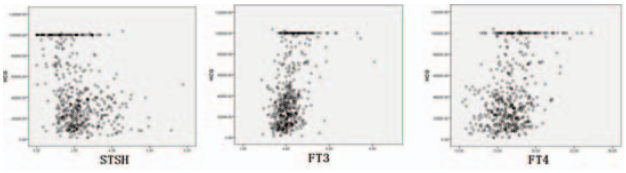

Figure 3. — Scatter diagrams of hCG with thyroid hormones.

Table 3. — *Thyroid hormones, TPOAb, and hCG levels in different stages of pregnancy (Mean ± SD).*

Group	n	FT3 (pmol/l)	FT4 (pmol/l)	TSH (mIU/l)	TPOAb	hCG
First 3 months	122	4.69 ± 0.71	18.34 ± 2.87	1.08	48.45	100000
Middle 3 months	240	4.09 ± 0.42	16.47 ± 1.96	2.13	36.15	28305
Last 3 months	128	3.86 ± 0.33	15.71 ± 2.01	2.39	37.70	20559
Control	51	4.69 ± 0.39	16.29 ± 2.32	1.89 ± 0.78	47.08 ± 13.7	
p		< 0.05	< 0.05	< 0.05	< 0.05	

higher than the manufacturer's value, whereas that during the last three months was slightly lower than the manufacturer's value. The high limit during the middle and last three months was lower than the manufacturer's value. During the first three months of pregnancy, the low and high limits were higher than the control. The low limit during the middle three months was higher than the control, but lower than the high limit. The low and high limits during the last three months were lower than the control. If the FT4, FT3, and TSH levels during the first three months and the TSH level during the last three months were evaluated according to manufacturer's values and control, the thyroid function of pregnant women may be misdiagnosed.

Variations of thyroid hormones and hCG levels in different stages of pregnancy

As shown in Figure 2, the serum FT3, FT4, and hCG levels decreased gradually throughout the entire pregnancy. During the first three months of pregnancy, the serum FT3 level was almost similar to the control; the FT4 level significantly higher. During the middle and last three months, the serum FT3 level was significantly lower than the control, whereas the FT4 level was almost similar or slightly lower. During pregnancy, the serum TSH level increased gradually, which was significantly lower than the control during the first three months and higher than the control during the middle and last three months of pregnancy.

The measurement results of thyroid hormones, TPOAb, and hCG levels are shown in Table 3. The overall differences among FT3, FT4, and TSH levels in the four groups were statistically significant (HFT3 = 193.58, HFT4 = 66.918, HTSH = 114.92; $p < 0.01$). The differences among FT3, FT4, and TSH levels between any two of the three stages of pregnancy were also statistically significant ($p < 0.01$). No significant difference of FT3 levels was noted between the control and first three months of pregnancy ($p = 1$), with significant difference from control to middle and last three months of pregnancy ($p < 0.01$). The difference of FT4 levels between the control and the first three months of pregnancy was significant ($p < 0.01$), whereas the differences from control to middle and last three months of pregnancy were not ($p = 0.996$, $p = 0.52$). A significant difference of TSH levels was observed from the control to the first and last three months of pregnancy ($p < 0.01$), with no significant difference from the control to the middle three months ($p = 0.08$). The overall differences of hCG levels among the three stages of pregnancy were significant (HhCG = 203.4, $p < 0.01$), with significant difference between any two of the three groups (HhCG = 203.4, $p < 0.01$). The TPOAb medians at each stage of pregnancy were within the reference ranges of the manufacturer's values (negative).

Correlation between hCG and thyroid hormones in pregnant woman

The scatter diagrams of hCG with thyroid hormones are shown in Figure 3. A negative correlation was observed between HCG and TSH (r = -0.367, $p < 0.01$), whereas a positive linear correlation was noted between hCG and FT3, as well as hCG and FT4, respectively (r = 0.431, $p < 0.01$; r = 0.35, $p < 0.01$).

Discussion

During pregnancy, a series of physiological changes occur in maternal thyroid, including thyromegaly and changes in the thyroid hormone level and autoimmune system, which are often caused by the TSH-similar regulatory effect of hCG and increased serum thyroid binding globulin. These changes cause the TSH level to decrease during and then increase after the early stage of pregnancy. The FT4 level increases during the early stage of pregnancy, followed by a decrease during the late stage [2]. This change has caused a perplexity in gestational diagnosis, treatment of thyroid diseases, and perinatal management. Stricker *et al*. [3]. used normal reference ranges of thyroid function for non-pregnant population to assess pregnant women, and found that approximately 3.6% of pregnant women with elevated TSH level are misdiagnosed and

Table 4. — *Reference ranges of FT3 (pmol/l), FT4 (pmol/l) and TSH (mIU/l) for pregnant women in different literatures.*

Authors	Index	First 3 months	Middle 3 months	Last 3 months	Indicating parameter
Gong *et al.*[12]	FT4	11-19	9.7-17.5	8.1-15.3	2.5th, 97.5 th
	n	224	240	211	
Lambert-Messerlian	TSH	1.05	1.23		Median
et al.[13]	FT4	14.16	13		Median
	n	300	300		
Haddw *et al.*[14]	TSH	1.0	1.29		Median
	n	1126	1126		
		378	375		
Yan *et al.*[15]	TSH	0.05-4.5	0.03-4.5	0.5-4.5	2.5th, 97.5 th
	FT3	3.6-5.6	3.65-5.2	3.5-5.2	2.5th, 97.5 th
	FT4	11.8-21.0	10.6-17.6	9.2-16.7	2.5th, 97.5 th
	n	168	168	169	
Panesar *et al.*[16]	TSH	0.8	1.09	1.56	Median
	FT3	3-5.7	2.5-4.1	2.1-4.2	2.5th, 97.5 th
	FT4	11.1-22.9	8.7-15.1	9.1-15.6	2.5th, 97.5 th
	n	343	343	343	
Springer *et al.*[17]	TSH	0.06-3.67			2.5th, 97.5 th
	n	4337			
Mannisto *et al.*[18]	TSH	0.07-3.5			2.5th, 97.5 th
	FT4	11-22			
	FT3	3.4-7	3.4-7	3.4-7	
	n	5805			
Karakosta *et al.*[19]	TSH	1.02	1.14		Median
	FT4	12.36-20.59	10.81-18.5		2.5th, 97.5 th
	FT3	2.83-8.28	1.99-8.14		2.5th, 97.5 th
	n	403	403		
Cotzias *et al.*[20]	TSH	0-1.6	1-1.8	0.7-7.3	2.5th, 97.5 th
	FT4	11-22	11-19	7-15	2.5th, 97.5 th
	FT3	4-8	4-7	3-5	2.5th, 97.5 th
	n	335	335	335	
Moleti *et al.*[21]	TSH	0.71	1.0	1.2	Median
	FT4	16.7±2.4	14.8±1.9	13.5±1.8	
		(11.1-26.2)	(10.9-19.4)	(13.5-18.3)	Mean
	FT3	5.69±0.77	5.84±0.92	5.69±0.92	
		(3.85-8.31)	(3.23-7.54)	(2.92-7.69)	Mean
	n	143	215	137	
Marwaha *et al.*[22]	TSH	0.6-5	0.44-5.78	0.74-5.7	5th, 95 th
	FT4	12-19.45	9.49-19.58	11.32-17.7	
	FT3	1.92-5.86	3.2-5.73	3.3-5.18	
	n	107	137	87	
Stricker *et al.*[23]	TSH	0.946	1.021	1.14	Median
	FT4	10.48-18.49	9.53-15.68	8.63-13.61	5th, 95 th
	FT3	3.52-6.22	3.41-5.78	3.33-5.59	5th, 95 th
	n	575	528	501	

3.7% are diagnosed with decreased TSH level. Assessing maternal thyroid function based on reference values for specific thyroid function in pregnancy has become a consensus. However, this process is seldom conducted in clinical practice because of the lack of specific regional reference ranges with particular thyroid hormone level. Previous studies [4-12] found that the hCG level reaches the highest value during the middle and late period of the first three months of pregnancy, stimulating the TSH receptors, and promoting the increase of thyroxine level and decrease of TSH level. The TSH level during this period was 0.2 mIU/l to 2.5 mIU/l, lower than that in non-pregnant women. However, the TSH level is over 3.5 mIU/l during late pregnancy. In early pregnancy, the total triiodothyronine and total thyroxine levels increase. The FT3 and FT4 levels correspondingly increase, followed by a gradual decrease. The 95% confidence interval of the FT4 level was 30% lower than the reference range for non-pregnant women. Yan *et al.* [13]. reported on the specific reference ranges of thyroid function for pregnant women in an area with sufficient iodine nutrition in northern China. The results of their study are generally consistent with the present study.

Iodine nutrition level is an important factor that affects gestational thyroid function. The iodine nutrition levels in different regions are not the same. The reference ranges for thyroid hormones in normal pregnant women in China's coastal area was investigated in the current study. The results showed that the serum FT3 and FT4 levels in pregnant women decreases gradually from the first to the last three months of pregnancy. During the first three months, the FT3 level was almost the same with the control, whereas the FT4 level was significantly higher. During the middle and last three months, the FT3 level was significantly lower than the control, whereas the FT4 level was almost the similar or slightly lower. The serum TSH level increased gradually during the whole pregnancy. The TSH level during the first three months was significantly lower than the control, but higher during the middle and last three months. These results show that the serum FT4 and TSH levels significantly vary in the first three months of pregnancy, but with little changes for the FT3 level. During the middle and last three months, the changes for FT3 and TSH levels become more significant, but with little changes for FT4 level. Therefore, the variations of thyroid hormones reflect the overall trend of change in thyroid function during pregnancy. However, the changes in trends of the three thyroid hormones were not similar. This condition is helpful in making a correct determination on the test results for pregnant women at different stages.

This study showed that the low and high limits of FT3 during the first three months of pregnancy were higher than the manufacturer's value. The low and high limits of FT4 were higher than the manufacturer's value and the control; however, the low and high limits of TSH were significantly lower than the manufacturer's values and control. If the reference ranges for non-pregnancy provided by the laboratory or manufacturers were used for normal pregnant women, they may be misdiagnosed with hyperthyroidism. Likewise, when pregnant women with subclinical hypothyroidism, of whom the serum TSH level is mildly elevated but less than the high limit of reference range, may be misdiagnosed as normal. According to "Management of Thyroid Dysfunction during Pregnancy and Postpartum: an Endocrine Society Clinical Practice Guideline" [2], the less than normal serum TSH level during the early stage of pregnancy should not be used as basis for the diagnosis of hyperthyroidism. During first-half period of pregnancy, the TSH median was about 0.8 μU/ml, with low limit of 95% confidence interval, 0.03 μU/ml. The serum FT4 level in first three months of pregnancy was higher than the control, but decreased during the last three months of pregnancy, which was consistent in this study. The low and high limits of FT3 and FT4 during the middle and last three months of pregnancy are within the range of control and manufacturer's values. During the

last three months, the low and high limits of TSH were higher than the control and manufacturer's values. If the reference range of the control and manufacturer's values are used in normal pregnant women, they may be misdiagnosed with hypothyroidism.

In this study, the serum hCG level decreased gradually throughout the entire pregnancy, with a variation similar with TSH, but opposite with that of FT3 and FT4. This result was consistent with the physiological change basis of normal pregnancy. The correlation between hCG and TSH was very strong; however, correlations of hCG with FT3 and FT4 may be the indirect results of TSH variation, resulting to similar clinical manifestations, to a certain degree, of hyperthyroidism and hyperemesis gravidarum.

The reported reference ranges of thyroid hormones [13-23] are shown in Table 4. Slight differences among the reference ranges were noted, which may be due to the difference in race, iodine nutritional state, region, and research method. However, the variations of FT3, FT4, and TSH levels during the three stages of pregnancy were consistent, similar to the current study. The subjects of this study were from Quanzhou, a coastal city in Fujian, China. According to "iodine content of salt (draft)" (national standards of food safety, Ministry of Health of China, 2010), the urinary iodine content in Fujian has an appropriate level (100 g/l to 200 g/l): however, the data for Quanzhou were not found. The study lacked the iodine nutrition status survey, which will be conducted in the authors' further studies on the characteristics of thyroid function changes in pregnancy.

The thyroid hormone levels in normal pregnant women are different from those in non-pregnant women; significant differences were observed among the three different stages of pregnancy. Thus, it is not an advisable strategy to manage thyroid diseases during pregnancy according to the reference ranges of thyroid hormones for non-pregnant women. The establishment of specific reference range of thyroid hormones in pregnancy is helpful for the evaluation of the thyroid function change during pregnancy, as well as for an effective management of thyroid diseases, thereby ensuring a normal pregnancy. This study has established reference ranges of thyroid hormones in normal pregnant women in one of China's coastal areas, which were not only suitable to for the clinical practice for this area, but also for other areas with similar iodine nutritional status in the population.

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Vitamin B12 and folic acid status of term pregnant women and newborns in the Antwerp region, Belgium

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Summary

Objective: Descriptive study on maternal serum vitamin B12 and folic acid in term pregnancy and in umbilical cord blood that was performed in an inner city hospital with a mixed ethnic population in the region of Flanders in Belgium. *Materials and Methods:* A prospective cohort study that took place from April 1 until May 31, 2011. Plasma folic acid and vitamin B12 were measured in maternal and umbilical cord blood from all term uncomplicated deliveries in a single regional hospital. Data on age, previous obstetric history, ethnicity, nutritional intake, and use of vitamin supplements were registered. *Results:* Data were collected from 110 patients, mean maternal serum vitamin B12 was 243.9 pmol/l and mean folic acid level was 43.0 nmol/l. Using a cutoff of respectively 150 pmol/l for vitamin B12 and 7.1 nmol/l for folic acid, 13% of the women were classified as vitamin B12-deficient and 23% were deficient for folic acid. Vitamin B12 deficiency was only seen in autochthonous Belgian women. A correlation between the maternal and umbilical cord levels was noted (R = 0.7 for vitamin B12, R = 0.85 for folic acid), but none of the umbilical cord levels demonstrated deficiency. Number of previous pregnancies and intake of supplements had no influence. *Conclusion:* Pregnant women in Antwerp, Belgium, frequently show vitamin B12 and folic acid deficiency, although a correlation exists with lower umbilical cord levels, the present limited data did not demonstrate any case of deficiency in umbilical cord blood. The frequency is highest in the autochthonous population and is not influenced by intake of vitamin supplements.

Key words: Vitamin B12; Cobalamin; Pregnancy; Umbilical cord; Vitamins; Ethnicity; Folic acid.

Introduction

Vitamin B12 or cobalamin is a co-enzyme in folate metabolism which is crucial to cell multiplication in pregnancy. The rapidly dividing placental and fetal tissue result in an increased need for both cobalamin and folic acid in pregnancy. Although much has been written on the role of folic acid supplementation in the periconceptional period and the prevention of neural tube defects, much less is known on the effects later in pregnancy. There are no clear cut reference values for vitamin B12 status in pregnant women; it has been suggested that serum vitamin B12 should be at least 221 to 295 pmol/l and the levels have been shown to differ between racial/ethnic groups [1-3].

In this study we aim to describe the cobalamin and folate level in maternal serum and umbilical cord in uncomplicated term pregnant women in an ethnically mixed city population in western Europe.

Materials and Methods

This was a descriptive single center study performed at Antwerp University Hospital (Edegem, Belgium). From April 1 to May 31, 2011, all term pregnant women presenting with spontaneous term labour were asked to have a blood sample drawn for vitamin B12 and folic acid, both a venous maternal serum sample immediately after informed consent was obtained and a sample from the umbilical vein at delivery. The study was approved by the local ethics committee and all patients signed a written informed consent. Term pregnancy was defined as 37 weeks or later, and complications such as diabetes, hypertension, fetal growth retardation or any other maternal disorder necessitating special care during pregnancy (including bariatric surgery and a previous baby with a congenital malformation), were excluded. A short food questionnaire was presented including questions on previous pregnancy and delivery, birth weight of previous children, previous surgery, congenital anomalies in the family (specifically asking for neural tube defects), use of vitamin supplements during pregnancy, any medication, eating eggs, meat, fish, vegetables, fruits, and alcoholic beverage consumption. Patients were also asked for their self-identified ethnic group; possibilities included autochthonous Belgian, Moroccan, Turkish, Central African, Western European (other than Belgian), Asian and "other".

Vitamin B12 was analysed and folic acid in maternal serum was measured. A serum level below 150 pmol/l was considered vitamin B12 deficiency; lower than 7.1 nmol/l was defined as folic acid deficiency, both as defined by WHO [4].

Statistics were performed with the SPSS 20.0 package. The authors used mean, standard deviation, differences between groups were compared with Student's t- test, and significance accepted at $p < 0.05$. Spearman correlation analysis was used to check for correlation between maternal and umbilical cord values. Linear regression was performed to evaluate other factors such as nutrition and use of supplements.

Results

Of 110 women included in the study period, 90 (81.8%) completed the questionnaire. There were 54 (48.8%) primiparous and 56 (51.2%) multiparous women. Eighty-three women (75.6%) were using vitamin supplements.

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An only brand was used containing 2.7 micrograms of vitamin B12 and 800 micrograms of L-methyl folic acid. There were 56 (51%) autochthonous Belgian women, 26 (24%) Moroccan, eight (7%) Asian, eight (7%) Central African, ten (9%) Western European other than Belgian, and only two (2%) Turkish.

Maternal serum vitamin B12 and folic acid were available for 78 (71%) of women, patients with missing samples were almost all women having signed the informed consent, but delivery was completed before blood samples were taken, mainly during night time. Mean serum cyanocobalamin was 243.9 pmol/l (standard deviation 93.9 pmol/l, minimum 2 pmol/l, maximum 500 pmol/l), mean folic acid in maternal serum was 43.0 nmol/l (standard deviation 185.2 nmol/l); this extremely large standard deviation was due to one women having an extremely high level (1,168 nmol/l), excluding this outlier from further analysis led to a mean value of 13.5 nmol/l (standard deviation 12.9 nmol/l, minimum 2.9 nmol/l, and maximum 21 nmol/l). Vitamin B12 deficiency was noted in ten (13% of serum samples) women; folic acid deficiency in 18 (23% of serum samples); none of these women had hemoglobin < 10.5 gram/dl. All cases of vitamin B12 deficiency were autochthonous Belgian women. Twelve out of 18 (66.7%) cases with folic acid deficiency were also autochthonous Belgian.

Umbilical cord blood samples were available for 88 cases (80%). Mean serum vitamin B12 was 612.7 pmol/l (standard deviation 414.0 pmol/l, maximum 1,586 pmol/l, and minimum 172 pmol/l); mean serum folic acid on umbilical cord venous blood was 18.4 nmol/l (standard deviation 3.7 nmol/l, maximum 21 nmol/l, and minimum eight nmol/l). Correlation between maternal and umbilical cord levels was rather weak (Spearman correlation coefficient R= 0.7) for cobolamin, and strong for folic acid (R = 0.85). There were no umbilical cord samples below the cut-off for deficiency, neither for vitamin B12 or folic acid.

Significant factors in linear regression determining maternal serum levels for folic acid were not eating eggs (p = 0.001) and consuming alcohol at least three to four times weekly (p =0.001). For vitamin B12, alcohol consumption also was significant (p = 0.033), but no other factors reached significance. Using a vitamin supplement was not related to the maternal serum level of vitamin B12 nor to folic acid.

Discussion

Despite the fact that the majority of women in the present sample was using vitamin supplementation, deficiencies for both folic acid and vitamin B12 were frequent and not less so in those using vitamin preparations containing folic acid and cobalamin. This can both be due to biologic factors, such as interference with food or absorption, but it can also be due to bias in reporting: perhaps women who report using vitamins do not really take them.

Reduced folate and increased vitamin B12 have been related to preterm birth and intrauterine growth restriction [5,6]; the present data demonstrate that folate and vitamin B12 deficiency are very frequent in normal term pregnancies (none of the babies in this cohort had a birth weight below 2,700 grams, the fifth percentile in this population), and considerable variation exists in these normal term pregnant women. A physiologic gradual decline in the serum concentration of vitamin B12, reaching a minimum at 32 weeks and the progressively rising again to term has been found by Morkbak *et al.* [7], as a high maternal serum concentration of vitamin B12 seems to be related to more preterm delivery and fetal growth impairment supplementation, with higher doses than in the kind of preparation used by the present patients might be harmful. There were no cases of low values for folate or vitamin B12 in umbilical cord (i.e. fetal) serum, underlining the inaccuracy of maternal serum levels to predict any deficiency in the offspring.

No clinically validated normal values for vitamin B12 or folate in pregnancy, including cut off values necessitating supplementation have been published. The present data suggest that cut-off values such as used in the non-pregnant population should not be used as far as provision of factors to the fetus is concerned, despite the correlation between maternal and umbilical cord levels; the latter were always above the minimal required level, suggesting a fetal benefit in supply even in case of maternal deficiency.

The present data do not allow any comment on the relation with neural tube defects or any other complication of pregnancy as the authors include only normal term pregnancies and not early pregnancy.

The autochthonous Belgian population has the highest risk for deficiencies in both vitamin B12 and folic acid. This can be partly explained by alcohol consumption (none of the Moroccan women consumed alcohol); this is just one more argument against the use of alcohol in pregnancy. The authors can only hypothesize that differences in diet are responsible for this and the food questionnaire utilized in the present cohort was not detailed enough to reveal this.

Conclusion

Pregnant women in Antwerp, Belgium, frequently show vitamin B12 and folic acid deficiency, correlated to lower umbilical cord levels but not as strong to lead to insufficient levels in umbilical/fetal blood. The frequency is highest in the autochthonous population and is not influenced by intake of vitamin supplements, but is associated to alcohol use.

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Clinical characteristics and reproductive outcome following hysteroscopic adhesiolysis of patients with intrauterine adhesion - a retrospective study

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Summary

The authors performed a retrospective clinical analysis of 153 patients with intrauterine ashesion (IUA) who underwent hysteroscopic adhesiolysis. A follow-up office hysteroscopy was performed in all cases after three months. On follow-up hysteroscopy, 22 patients showed reformation of adhesions and required a repeat procedure. The primary risk factor for IUA was uterine curettage associated with pregnancy termination. The follow-up study revealed that the rate of pregnancy after IUA treatment was 51%. The conception rate in women who had reformation of IUA was significantly lower than that of women who had a normal cavity following adhesiolysis. Therefore the authors conclude that prevention is more important than therapy in IUA. Increasing education about avoiding curettage is necessary to reduce the incidence of IUA. Outreach is particularly important for older women with less education. However, hysteroscopic adhesiolysis for IUA is a safe and effective method of choice for restoring menstrual function and fertility.

Key words: Intrauterine adhesion; Hysteroscopic adhesiolysis; Reproductive outcome; Adhesion prevention.

Introduction

It has been more than one century since Heinrich Fritsch first described intrauterine adhesion (IUA) caused by trauma to the uterine cavity [1]. In 1948, Joseph G. Asherman published a series of papers to describe the frequency, etiology, symptoms, and roentgenologic picture of this condition, and Asherman syndrome has been used to describe the disease ever since [2]. IUA is a consequence of trauma to the endometrium, which causes partial or complete obstruction in the uterine cavity and/or the cervical canal, resulting in conditions such as menstrual abnormalities, infertility, recurrent pregnancy loss, fetal intrauterine growth retardation, abnormal placenta development, and other complications related to conception. Universally, the incidence of IUA is increasing, mainly from curettage during induced, incomplete, or missed abortions, postpartum hemorrhage, and genital tuberculosis [3]. Any operation in the uterine cavity can cause IUA, therefore only necessary operations should be performed. Currently, hysteroscopy is the method of choice to diagnose, treat, and follow patients with intrauterine adhesions. In China, IUA incidence, detected by hysteroscopy, has increased and presents with diverse manifestations. The treatment of severe IUA is still puzzling for clinicians. Bearing in mind the triad of poverty, ignorance, and disease, and the vicious cycle thus generated [4], this paper aims to outline the clinical characteristics of women presenting with IUA and their reproductive outcome for better prevention, diagnosis, and treatment of IUA.

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Materials and Methods

The authors retrospectively enrolled the patients with IUA who attended the in-patients of minimally invasive center of Obstetrics and Gynecology Hospital of Beijing during a six-year period between June 2005 and June 2011. The inclusion criterion for this study was hysteroscopically diagnosed IUAs. In all patients, a comprehensive infertility workup was performed including: a tubal patency test, pelvic ultrasonography, husband semen analysis, and serum hormone measurements (FSH, LH, prolactin, estradiol, progesterone, and androgen) on the second to fifth day of the cycle or at a randomly chosen time in patients with amenorrhea. Any patients with abnormal test results that may have been responsible for reproductive failure were excluded from the study.

Hysteroscopic adhesiolysis was performed under general anesthesia by an operator experienced with operative hysteroscopic procedures under laparoscopy or ultrasound using a monopolar knife. At the end of the adhesiolysis, a T-shaped intrauterine contraceptive device (IUCD) was inserted as a stent into the uterine cavity and hormone treatment was begun, consisting of estradiol valerate at a dose of four mg/day for 21 days, with the addition of medroxyprogesterone acetate at a dose of ten mg/day in the last ten days of estrogen treatment. Antibiotics were used after the operation. After three months, a second follow-up hysteroscopy was performed in the early proliferative phase of the menstrual cycle in those patients who were menstruating. If reformation of adhesions had occurred, a repeat adhesiolysis procedure was performed during the follow-up procedure. The IUCD was removed during the follow-up hysteroscopy once the presence or absence of adhesion had been determined. If a repeat adhesiolysis procedure was performed, a second IUCD was inserted into the uterine cavity and hormone treatment was repeated. All patients had a follow-up examination and 22 patients required a second adhesiolysis. No patient underwent more than two procedures. In patients who remained amenorrheic in spite of hormonal treatment, a diagnostic hysteroscopy was also performed three months after the initial hysteroscopic adhesiolysis. When adhesions were absent at

Table 1. — *Subject characteristics of patients with IUA.*

Item	Mininum	Maximum	mean	std. Deviation
Age (years)	22	43	32	4.4
Education (years)	9	20	13.9	2.9
Gestation (n.)	0	7	2.2	1.6
Parity (n.)	0	5	0.2	0.5
Operation time (minutes)	10	120	38.9	21.5

Predisposing factor in the patients of IUA	number (n)	Percentage (%)
Trauma to a gravid uterine cavity		
Dilatation and curettage	132	86.6
Evacuation for hydatidiform mole	2	1.3
Infection after labor	2	1.3
Trauma to non-gravid endometrium		
Polypectomy	1	0.65
Resection of septum	1	0.65
Myomectomy	1	0.65
Unexplained	14	9.2

the second hysteroscopy, the patients were encouraged to resume their efforts to conceive.

A follow-up by telephone was conducted during July 2012 to learn the reproductive outcome and the amount of time between operation and conception in the enrolled patients. The study included 153 patients. According to the American Fertility Society (AFS) score system published in 1988 [5], the patients were divided into mild, moderate, and severe groups based on the severity of IUA.

Statistical analysis was performed using SPSS 18.0 version software. For continuous variables, t tests were used for between group comparisons and were expressed as means ± standard deviation (SD); for categorical variables; $\chi 2$ tests were used for between group comparisons. The α-level was set at 0.05.

Results

The mean age of patients with IUA was 32.0 years, with a range between 22 and 43 years of age. The mean time of gestation was 2.2, and the parity was 0.2. The mean duration of education was 13.4 years, and the mean duration of operation time was 38.9 minutes (Table 1). The most significant contributing factor to an IUA diagnosis was a history of dilatation and curettage for uterine evacuation, either for a spontaneous or induced abortion or postpartum haemorrhage. Other contributing factors included: a history of polypectomy, resection of the septum, and myomectomy. No apparent predisposing factors were found in 14 of the patients enrolled in the study (Table 1). Based on their reproductive outcome, the patients were divided into a group that successfully conceived and a group that did not successfully conceive. The patients able to conceive were significantly younger (31.0 *vs*. 32.9 years, $p = 0.038$) than those unable to conceive and more educated (14.3 *vs*. 13.4 year, $p = 0.001$). There was no significant difference in the time of gestation or parity and the operation duration between the two groups.

Table 2. — *Clinical presentation*.*

Presentation	Number	Percentage
Primary infertility	18	11
Secondary infertility	32	21
Secondary amenorrhea	15	10
Hypomenorrhea	73	48
Abnormal mass in uterine cavity	5	3
Malformation of uterine	7	5
Lower abdominal pain	2	1
Ectopic pregnancy	1	1

* more than one kind of presentation in some patients.

Table 3. — *The relationship between the main manifestation and disease score.*

Classification	Number n (%)	Abnormal menstruation n (%)	Infertility n (%)	*p*
Mild	10 (7.2)	4 (40)	6 (60)	0.017#
Moderate	78 (56.5)	34 (43.6)	44 (56.4)	0.007*
Severe	50 (36.2)	40 (80)	10 (20)	

the mild group compared with the severe one
* the moderate group compared with the severe one

Patients presented with diverse clinical manifestations are shown in Table 2. There were 13 (8.5%) patients in the mild degree group, 86 (56.2%) in the moderate group, and 54 (35.3%) patients in the severe degree group. Menstrual abnormalities and infertility were present in 90% of cases overall. Among the 13 patients in the mild group, four (40%) patients presented with abnormal menstruation and six (60%) with infertility. In the moderate and severe groups, the number of patients presenting with abnormal menstruation or infertility were 44 (43.6%) and 34 (56.4%), and 40 (80%) and ten (20%), respectively (Table 3). In the severe group the main clinical presentation was abnormal menstruation, and in the moderate and mild groups it was infertility.

A follow-up by telephone was conducted to determine the menstrual pattern, conception rate, time between treatment and conception, and the reproductive outcome for each patient. The numbers of women with amenorrhea, hypomenorrhea, or normal menstruation before and after surgery were 15/115/23 and 2/74/77, respectively. Amenorrhea and hypomenorrhea prior to surgery were improved in 67 out of 130 patients (51.5%). Table 4 shows the correlation between conception and menstrual pattern before and after adhesiolysis.

The conception and reproductive outcomes of all 153 patients after hysteroscopic adhesiolysis are given in Table 5. Among 153 women, 78 (51%) achieved pregnancy. In the 22 patients with reformed intrauterine adhesions at the follow-up hysteroscopy, only four conceptions (18.2%) occurred despite further adhesiolysis. Among 131 women with normal cavities at the follow-up hysteroscopy, 74 conceptions (56.5%) occurred ($p < 0.05$). The mean time in-

Table 4. — Menstrual pattern before and after hysteroscopic adhesiolysis and relationship to conception rate.

Menstrual pattern before treatment (n = 153)	Menstrual patterns after treatment			
	Amenorrhea (*n* = 2)	Hypomenorrhea (n = 74)	Normal menses (n = 77)	Conception rate (%)
Amenorrhea (n = 15)	0/2	4/10	1/3	5/15 (33.3%)
Hypomenorrhea (n = 115)	0	29/63	30/52	59/115 (51.3%)
Normal menses (n = 23)	0	0/1	14/22	14/22 (63.6%)
Conception rate (%)	0	33/74 (44.6%)	45/77 (58.4%)	78/153 (51.0%)

Table 5. — Conception and reproductive outcomes in 153 patients after hysteroscopic adhesiolysis with different grades of adhesion.

No.	Variable	Mild (*n* = 13) (%)	Moderate (*n* = 86) (%)	Severe (*n* = 54) (%)
1	Conception	7/13 (53.8)	49/86 (60)	21/54 (38.9)
2	Spontaneous miscarriage	1/7 (14.3)	6/49 (12.2)	3/21 (14.3)
3	Ongoing pregnancy	3/7 (42.9)	13/49 (26.5)	2/21 (9.5)
4	Live birth	3/7 (42.9)	30/49 (61.2)	16/21(76.2)

terval between treatment and conception was 15.9 months. Of the 78 pregnancies, 49 (62.8%) were live births, ten (12.8%) had spontaneous miscarriages, and 19 (24.4%) were pregnant at the time of follow-up (three pregnancies were less than 12 weeks and 16 pregnancies were more than 12 weeks at the time of follow-up). Forty women underwent cesarean sections to give birth; six of those patients had abnormal placentas. In those six cases, one case was placental adhesion and two cases were placental accreta, both patients had postpartum hemorrhaging, and one patient lost her uterus. The remaining three cases were placental previa in which one case achieved termed birth, while the other two cases were preterm deliveries at 35 weeks and 32 weeks, respectively. Fourteen patients attempted assisted reproductive techniques, and of those, five patients had term births and two patients had abortions. The mean time interval between operation and conception in these cases was 29 months.

Discussion

The patients enrolled in the study generally had lower levels of education and were older women. IUA presentation was more severe the more times the patients had undergone curettage and the lower their education level. It is possible to educate women about contraception to avoid unplanned pregnancy and reduce incidence of curettage. In China, attitudes and behavior are changing, on one hand sex is becoming more open and sexual behavior is increasing, and on the other hand unplanned pregnancy is increasing. Women also delay planned conception because of the rapid pace of life and the competition of work; both of which increase the opportunity of curettage for accidental conception.

The main complaint of patients with IUA was abnormal menstruation or infertility, and there was some relationship between the severity score and clinical manifestation. This relationship may have been caused by the classification criteria. The AFS score emphasizes the pattern of menstruation but leaves out the symptom of infertility. Therefore, in infertility patients, it is desirable to perform hysteroscopy to evaluate the capacity of the reproductive system. For patients who had normal menstruation, it was difficult to explain the occurrence of IUA. It may have been caused by chronic reproductive system infection or latent tuberculosis infection. Furthermore, chronic tuberculosis is difficult to diagnose. Another symptom is infertility caused by obstruction of the tubal ostia or endocervix. Patients can have subclinical recurrent abortion caused by the smaller uterine cavity size, poor endometrial receptivity, the defect of endometrium, myometrial fibrosis, and reduced uterine blood flow [6]. The present analysis supports that trauma to a gravid uterine cavity is the main cause of Asherman's syndrome [7]. One of the possible explanations for the gravid uterus being a major predisposing factor to Asherman's syndrome is the low estrogen status at the time of the operation or immediately following.

Modern treatment of IUA has focused on two areas: the first is the actual management of the adhesions, and the second is preventing adhesion reformation. In severe cases, it may be necessary to perform concurrent ultrasonography to facilitate passage through the endocervical canal and internal os into the cavity. Simultaneous laparoscopy may also be valuable for guiding adhesiolysis. The most effective therapy is combination of post-hysteroscopical IUD and administration of artificial menstruation period [6]. Different techniques for hysteroscopic adhesiolysis have been described. Hysteroscopic scissors [8] or laser treatment [9] has been used to divide adhesions. Hysteroscopic resection with a monopolar probe was also found to be efficient [10, 11]. In the present study, the authors used a monopolar knife to divide adhesions, which is effective, safe, and less expensive in a limited resource setting. The concurrent laparoscopy in this study to confirm the tubal patency and to rule out other pelvic pathology also helped to know the end point of adhesiolysis by observing the transillumination. There were no cases of uterine perforation in this series, which

may be due to concurrent laparoscopy during the procedure. There were two cases (1.3%) of complication of transurethral prostatic resection (TURP) in 153 patients. After the administration of sodium chloride, furosemide, and aminophylline, the symptom was eliminated. The mean duration of the hysteroscopic adhesiolysis procedure is reported to vary between ten and 45 minutes [12, 13]. In the present series, the mean operating time was 38.9 minutes, and the difference in the mean operating time for mild, moderate, and severe Asherman's syndrome was not statistically significant.

Serum from the areas of freshly dissected scars can promote scar reformation. Thus, a non-reactive uterine stent is placed to keep the uterine walls apart during the initial postoperative healing phase. Polishuk *et al.* [14] reported that by following adhesiolysis with IUD placement, the rate of adhesion reformation was only 10%. In contrast, in a prior series of their patients treated without an IUD, the recurrence rate was >50%. Several other methods for reducing re-formation of adhesions have been advocated, including the use of amnion around a balloon catheter, the use of a spray gel adhesion barrier, and early intervention after electrocautery lysis of adhesions [6, 15, 16]. However, none of these methods have sufficient data to recommend them at this time. Therefore, in the present study the authors used an inert IUD kept in situ for 90 days and reported no complications. A combination of estrogen and progestin therapy has been successfully used [17, 18]. However, they preferred giving estradiol valerate at a dose of four mg/day for 21 days, with the addition of medroxyprogesterone acetate at a dose of ten mg/day in the last ten days of estrogen treatment.

It has been reported that improvement of menstrual flow after hysteroscopic adhesiolysis ranges from 52.4 to 74.2% [12]. In the present series, the improvement of menstrual function was 70.6% (53 out of 75), which was similar to a recent report [17]. The authors also found that there was no significant association between conception rate after adhesiolysis and preoperative menstrual pattern. Extensive review articles consistently find the same numbers, with live birth rates of 32 to 76%. In most series, the more extensive the adhesions, the lower the pregnancy and live birth rates. For those patients who achieve pregnancy, there is a significant risk of complications including placenta accreta [16]. Yu *et al.* [17] in a recent study of 85 patients with 109 operative procedures, found that women who remained amenorrheic had a significantly lower chance of conception; 18.2% versus 50%. Similarly, the conception rate for women with a normal cavity at follow-up hysteroscopy was 59.1% versus 11.8% for those who had re-formation of adhesions. In the present analysis, 78 (51%) patients out of 153 patients became pregnant. Among those, ten had abortions, and 49 patients had live deliveries; 16 patients were more than 12 weeks pregnant at follow-up. The mean time interval between operation and conception was 15.9 months. Of the live deliveries, the cesarean rate amounted to 81.6%. Patients with more severe adhesions had a higher cesarean rate. Women who successfully became pregnant were younger and more educated. Therefore, it is important to increase outreach to women with a lower education levels and provide pregnancy planning at younger ages. Furthermore, for patients with IUA, it is essential to emphasize supervision during pregnancy to avoid complications.

Re-adhesion after adhesiolysis is the main factor which effects the outcome of reproduction in IUA patients [17]. The mechanism of adhesion formation after operation in the abdominal cavity has been reported [17, 19]. However, research in the field of operations in the uterine cavity is rarely seen. It is unknown which cytokines or genes take part in the formation of uterine adhesions. Molecular research in wound healing of the skin may supply some hints. This work indicates that miRNA plays an important role in all stages of wound healing of skin [20]. Chronic wounds are a major health burden and developing newer and more effective treatments has therefore become a necessity. Knowledge of miRNA function in the regulation of wound healing and developing improved miRNA modulation techniques in the skin will help translate this knowledge into more effective therapies.

In conclusion, IUA was readily seen in the women with lower education levels. Because of the burden of life and work, women may delay planned pregnancies. So it is essential to educate woman about the hazards of curettage and the decline of reproductive ability with aging. Hysteroscopic adhesiolysis is a safe and effective procedure for the restoration of normal cavity and menstruation. The choice of anti-adhesions still requires large, rigorous, and multi-center research trials to verify best practices. Future research should focus on the prevention of re-adhesion after adhesiolysis and supervision for pregnant woman after adhesiolysis.

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Implications of premature ovarian failure on bone turnover markers and bone mineral density

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Summary

Introduction: Premature ovarian failure (POF) is the cessation of ovarian function before the age of 40. The loss of ovarian function, whether premature or not, has an overwhelming impact on female skeletal health, leading to an increased risk of developing osteoporosis because of the lengthened time of exposure to reduced estrogen. The objective of this study was to compare the implications of premature ovarian failure on bone turnover markers and bone mineral density in patients under the age of 40. *Materials and Methods*: Sixty-one patients with a diagnosis of POF were selected for this prospective study. Patients were divided into two groups according to age, patients < 30 years old (n = 30), and patients ≥ 30 years old (n = 31). *Results:* Between the two age sub-groups (< 30 and ≥ 30 years old), there was a significant difference in menopause rating scale (MRS), lumbar spine t-score, N-telopeptides crosslinks (NTx), and serum bone specific alkaline phosphatase (bALP) between the two age groups (10.93 ± 7.79 vs 17.38 ± 8.62; -1.84 ± 1.47 vs -1.06 ± 0.93; 58.80 ± 21.32 vs 41.1 ± 11.37; 48.99 ± 42.16 vs 23.76 ± 10.08, respectively). *Conclusion:* It is apparent that bone mineral density (BMD) is commonly less in women with POF than normal healthy women. Therefore, measurement of BMD is warranted. At this time, it is not clear how often the tests should be carried out to evaluate BMD. Further prospective studies are required to establish guidelines. However, it seems reasonable to monitor women with POF yearly for the presence of any endocrine dysfunction and to assess BMD at periodic intervals.

Key words: Premature ovarian failure; Bone mineral density; Osteoporosis; Bone turnover markers.

Introduction

Premature ovarian failure (POF) is the cessation of ovarian function before the age of 40. Recently, POF has been under the spotlight, as more young women have elevated levels of follicle-stimulating hormone (FSH) and decreased circulating levels of estrogens when they present with absent or irregular menses or infertility [1]. There are no accurate estimates of the prevalence of POF. An estimate based on a considerable number of studies showed that 0.3% of women of reproductive age has POF [2].

The cause of POF is still unclear. There is not a single cause that is predominant, which can cover a large percentage of the POF cases. As the ability to treat malignancies successfully with chemotherapy and irradiation increases in the field of oncology, the number of young women presenting with transient or permanent POF increases as well. There are also several genetic causes for POF. Karyotypic abnormalities, single gene mutations, and complex multifactorial polygenic inheritance account for some causes.

The loss of ovarian function, whether premature or not, has an overwhelming impact on female skeletal health, leading to an increased risk of developing osteoporosis because of the lengthened time of exposure to reduced estrogen. Currently, it is estimated that one in every two Caucasian women will experience an osteoporotic fracture during her lifetime, which increases the morbidity rate and cause a considerable economic burden in the aging female population [3]. Consequently, most studies have been conducted in aging postmenopausal women, while fewer studies focused specifically on changes in bone mineral density (BMD) and bone turnover markers in young women with POF.

The objective of this study was to compare the implications of premature ovarian failure on bone turnover markers and bone mineral density in patients under the age of 40.

Materials and Methods

Sixty-one patients, who were admitted to the Infertility Clinic of Istanbul University School of Medicine, with a diagnosis of POF were selected for this cross-sectional study. Patients were divided into two groups according to age, patients < 30 years old (n = 30) and patients ≥30 years old (n = 31). Approval of the ethics committee of Istanbul University and informed consent from all participants were obtained prior to the treatment.

Inclusion criteria were as follows: (1) at least four months of amenorrhea; (2) two FSH values of > 40 mIU/ml obtained at least one month apart; (3) Estradiol (E2) value of < 40 pg/ml; (4) age under 40 years old. Exclusion criteria were pre-existing hepatic, renal, metabolic or bone diseases or the use of drugs in the past three months that could influence bone metabolism, such as oral contraceptives and hormone replacement therapy.

For each study subject, the authors obtained information on age, height, weight, sexual activity level, obstetric-gynecological history (para/abortus, age at menarche, regularity of menstrual periods, cessation of periods, use of hormone replacement), med-

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ical history (previous chemotherapy or radiotherapy, pelvic surgery, existence of systemic diseases as thyroid, hyperprolactinemia, rheumatoid arthritis, hypertension, malignancy), and family history (POF in either mother or sister). Each patient underwent gynecologic examination, Pap smear and vaginal ultrasound. Body mass index (BMI) for each patient was calculated as weight (kg) divided by the square of the height (m^2). Menopause rating scale (MRS II), developed by Potthoff *et al*., was utilized to measure the severity of menopause symptoms [4]. Complete blood count, hormone profile (including luteinizing hormone (LH), FSH, E2, progesterone, prolactin (PRL), thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), dehydroepiandrosterone sulfate (DHEA-SO4), sex hormone binding globulin (SHBG), testosterone, thyroid peroxidase antibody (TPOAb), antithyroglobulin antibody, liver and kidney function, blood glucose test, and blood lipid tests were ordered for each patient. Karyotyping was performed in patients under the age of 30.

BMD Measurements

Both lumbar spine (L2-L4) and the total hip were chosen as measurement sites for BMD. BMD was measured by dual X-ray absorptiometry in the Osteoporosis Screening and Treatment Clinic of Istanbul University School of Medicine.

Blood biochemical analyses

Blood samples were obtained between 8:00-10:00 a.m. after an overnight fasting. Samples were centrifuged at 2,000 rpm for ten minutes at room temperature. The separated sera were stored at −80°C until analyzed.

Complete blood test count was performed on an autoanalyzer. Hormone profile, TPOAb ,and antithyroglobulin antibody were assayed by electrochemiluminescence immunoassay.

Serum bone-specific alkaline phosphatase (bALP) and procollagen type I C-peptide (PICP) were measured as markers of bone formation. BALP was assayed using an enzyme immunoassay kit and PICP was measured with two-step enzyme immunoassay. The interassay coefficients of bALP ranged from 5.0% to 7.6%. The intra-assay coefficients of bALP ranged from 3.9% to 5.8%. The interassay and intra-assay coefficients of PICP were 6.6% and 5.4%, respectively.

Cross-linked N-telopeptides of type I collagen (NTX) was measured as a marker of bone resorption. It was determined by enzyme immunoassay kit. The interassay coefficients of NTX ranged from 3% to 5%. The intra-assay coefficients of NTX ranged from 5% to 8%.

Statistical analysis

All values were expressed as mean ± SD. The corresponding data from BMD and bone turnover markers measurements were compared statistically using the Mann-Whitney U test. To determine the potential association of BMD and bone turnover markers, bivariate correlation analysis was used first to calculate Pearson's correlation coefficients. Multiple linear regression analysis was used to estimate which parameter was the most important index to predict BMD. BMD at the lumbar spine and the total hip were chosen as the dependent variable separately. A SPSS 15.0 software package was used for all statistical procedures; $p < 0.05$ was considered significant.

Results

All participants of the study were Caucasian. The mean age of the patients at the time of the study was 27.9 years (ranging 16 to 38). Out of 61 patients, 54 patients (89%) were diagnosed with POF and seven patients (11%) with hypogonadotropic hypogonadism. In 54 POF patients, seven were diagnosed with Turner syndrome (gonadal dysgenesis), while two patients experienced surgical menopause. 35% of the patients who were younger than 30-years-old were either married or had a partner, while 86% of the patients who were 30 years of age or older were either married or had a partner.

Table 1. — *Characteristics of patients.*

	Age		Levene's test		t-test
	< 30 n = 30	≥ 30 n = 31	F	*p*	*p*
Weight (kg) [a]	59.03 ± 12.14	65.33 ± 13.13	0.051	0.822	0.059
BMI (kg/m^2) [a]	23.13 ± 4.46	24.6 ± 5.21	0.206	0.652	0.221
MRS (0-44) [a]	10.93 ± 7.79	17.38 ± 8.62	0.357	0.552	0.004
BMD lumbar spine (t-score) [a]	-1.84 ± 1.47	-1.06 ± 0.93	5.465	0.023	0.021
BMD total hip (t-score) [a]	-0.83 ± 1.30	-1.07 ± 1.15	0.225	0.637	0.489
NTX (nmol) [a]	58.80 ± 21.32	41.1 ± 11.37	5.403	0.024	0.000
bALP (U/l) [a]	48.99 ± 42.16	23.76 ± 10.08	10.990	0.002	0.004
PICP (ng/ml) [a]	221.19 ± 136.93	171.65 ± 95.68	1.250	0.269	0.147
FSH (mIU/ml) [a]	58.79 ± 42.34	66.44 ± 34.99	2.024	0.160	0.450
LH (mIU/ml) [a]	27.56 ± 25.87	35.58 ± 20.75	0.625	0.433	0.209
E2 (pg/ml) [a]	22.80 ± 25.76	20.23 ± 11.86	1.721	0.195	0.631
Prolactin (ng/ml) [a]	13.52 ± 6.66	13.46 ± 8.89	1.265	0.266	0.978
TSH (mIU/l) [a]	2.57 ± 3.04	1.83 ± 0.991	0.536	0.468	0.242
DHEA-SO4 (µg/dl) [a]	170.39 ± 90.10	180.06 ± 72.89	2.918	0.098	0.736

[a] Values are expressed as mean ± SD.

Table 2. — *Correlation of BMD, BMI, and bone turnover markers.* [a]

	BMD (Total hip)	BMI	NTX	BALP	PICP
BMD (lumbar spine)	0.511	0.313	-0.665	-0.595	-0.340
BMD (total hip)		0.333	-0.098	-0.327	-0.009
BMI			-0.373	-0.304	-0.197
NTX				0.553	0.404
bALP					0.359

[a] Spearman correlation was used.

Between the two age sub-groups (< 30 and ≥ 30 years), there was not a significant difference in weight, BMI, period of menopause, total hip t-score, PICP, FSH, LH, E2, prolactin, TSH, and DHEA-SO4 (Table 1). There was a significant difference in MRS, lumbar spine t-score, NTX, and bALP between the two age groups (10.93 ± 7.79 vs 17.38 ± 8.62; -1.84 ± 1.47 vs -1.06 ± 0.93; 58.80 ± 21.32 vs 41.1 ± 11.37; 48.99 ± 42.16 vs 23.76 ± 10.08, respectively) (Table 1).

Table 2 presents correlations between BMI, BMD (lumbar spine and total hip t-scores), and bone turnover markers (NTX, BALP, and PICP). There was a significant negative correlation between lumbar spine t-score and NTX (p = -0.665), between lumbar spine t-score BALP (p = -0.595) and between lumbar spine t-score and PICP (p = -0.340). A significant negative correlation was also observed

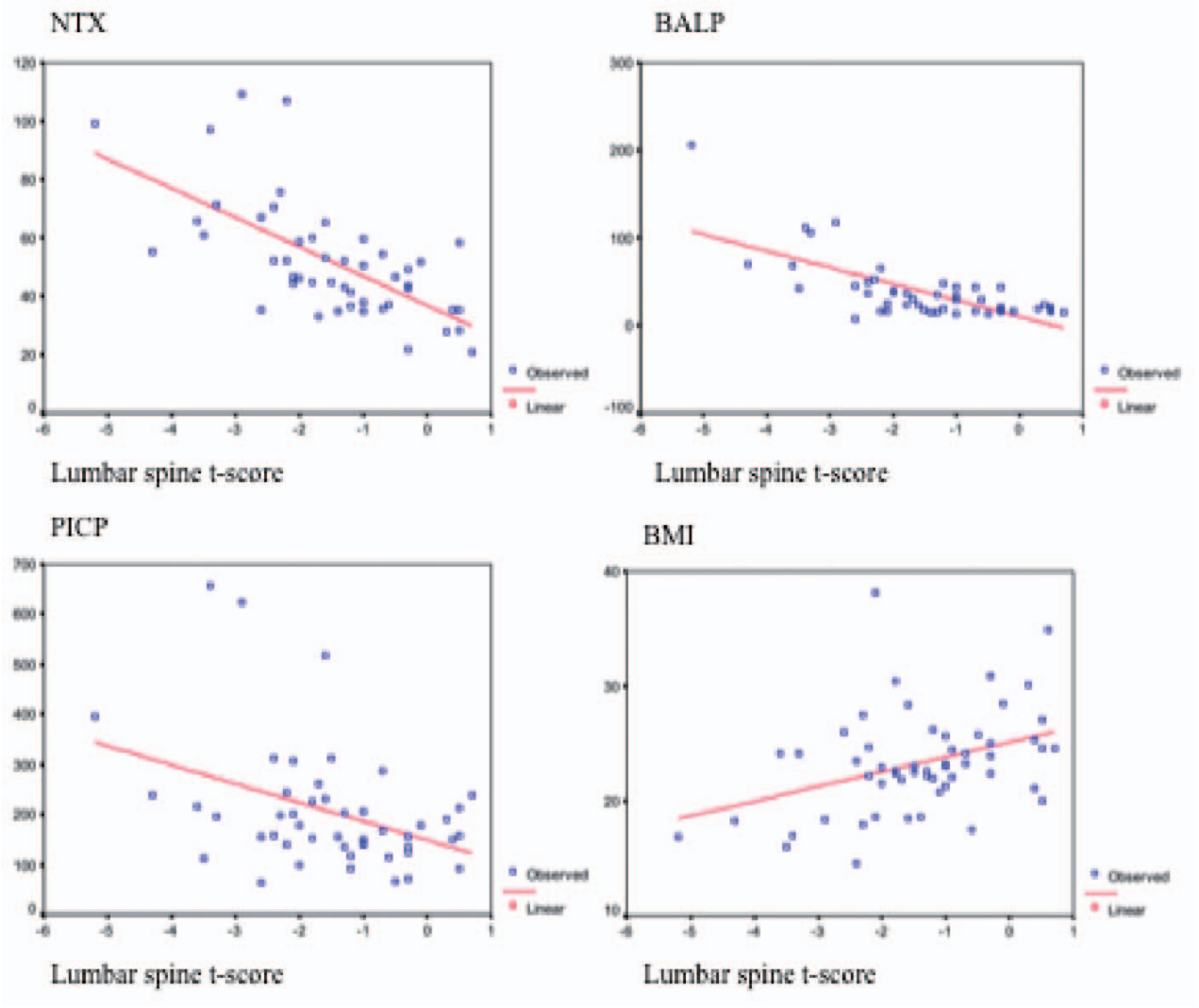

Figure 1. Regression analyses between BMD lumbar spine and NTX, bALP, PICP, and BMI.

between total hip score and bALP (p = -0.327). BMI had a significant negative correlation to NTX and bALP (p = -0.373; p = -0.304, respectively).

When lumbar spine t-score as the BMD measurement was further analyzed, there was a negative correlation between lumbar spine t-score and NTX, bALP, and PICP, while there was a positive correlation between lumbar spine t-score and BMI (Figure 1).

Discussion

Although there is a large body of literature examining BMD among postmenopausal women and a moderate body of literature examining BMD among premenopausal women, fewer studies have monitored BMD in women with POF. According to the present authors' limited knowledge, this study is the first to compare bone turnover markers and BMD in women with POF in two age groups. Thirty years of age was chosen as a benchmark, because it is a well-established fact that peak bone mass is reached around this age. Therefore, the authors wanted to classify and evaluate women with POF that are under 30 and above 30 years separately and compare the outcomes. This study revealed that women under the age of 30 had higher levels of bALP and NTX. On the other hand, MRS was higher in women who were 30 years of age or older.

BMD assessment measured by DXA is currently the "gold standard" for the diagnosis of osteoporosis. According to the World Health Organization (WHO) criteria, osteoporosis is defined by a t-score of -2.5 or below [5]. In the present study, all participants were osteoporotic since their t-score was well below -2.5. Lately, bone turnover markers, which reflect either bone formation or bone resorption cell activities, also play a significant role in assessing

BMD. For postmenopausal women, negative correlations are frequently reported between bone turnover markers and lumbar spine t-score, especially for bALP [6-10] and NTX [6-9, 11-15]. In premenopausal women, NTX is usually the only marker, which is significantly correlated with BMD [6, 7, 11, 13, 16]. The present study revealed a correlation between BMD and bone turnover markers. BALP, NTX, and PICP significantly correlated with lumbar spine t-score, while bALP significantly correlated with total hip t-score. In addition, the present study showed a significant negative correlation between BMI and NTX and between BMI and bALP, which is a further affirmation of the well-known correlation between BMI and BMD [17].

In cross-sectional analyses, bone resorption markers are consistently higher in postmenopausal, while bone formation markers are more variable [18-21]. Higher levels of bone turnover markers have been shown in many, yet not in all studies [19, 22-24]. Only a few number of studies have measured bone turnover markers in perimenopausal women or women with POF. In a cross-sectional analysis, NTX was measured in 2,375 participants who were either premenopausal or early perimenopausal [25]. NTX was slightly higher in the perimenopausal. However, the differences were not significant. In a very recent study, no individual bone turnover marker's increase was predictive for perimenopausal BMD loss [26]. In the present study, bALP and NTX were higher in women with POF who were younger than 30 years of age compared with women with POF who were 30 years of age or older. Reduced estrogen might have a more significant impact on BMD in younger women. On the other hand, it is a well-established fact that bone turnover markers increase significantly during puberty [27]. Since women who are younger than 30 years are still in late adolescent stage, their bone turnover marker levels might still be fluctuating.

When evaluating bone turnover markers, there is also the issue of reference intervals. Presently, studies on reference intervals are very limited and there are no established criteria. Blumsohn *et al.* compared bone turnover markers of healthy premenopausal women living in five different European countries and found no significant difference [28]. However, studies on healthy premenopausal women conducted in UK [29], USA [30], France [31], and Italy [32] reported different reference intervals. These inter-country variations may be related in part to factors such as BMI, alcohol consumption, or smoking as suggested by Glover *et al.* [33]. These authors conducted a study, including 637 healthy young premenopausal women from UK, France, Belgium, and the USA and established reference intervals for some of the bone turnover markers. The reference interval was 5.15–8.68 ng/ml for bALP and 9.22–24.8 nmol BCE/mmol for NTX. Unfortunately, there are no reference intervals regarding PICP as of now. In the present study, both bALP and NTX levels of all participants were well over the suggested reference interval levels.

In the present study, MRS score was significantly higher in women who were 30 years of age or older. The rate of marriage or being in a relationship was also higher in this sub-group. In general, perimenopausal and postmenopausal women present significantly higher rates of menopausal symptoms when compared to premenopausal women and subsequently, MRS scores usually increase in relation to age and the menopausal stage [34]. Moreover, being diagnosed with POF can be an upsetting diagnosis and women often express depression, anxiety, loss, and sadness. In addition, women with POF who are married or in a relationship may be facing the traumatic impact of potential infertility in their relationships or marriages. As quality and expectancy of life increase, even women in natural menopause stages care more about potential health issues they might face during this period. Facing menopause in early ages is devastating for young women with POF. Therefore, consulting patients is extremely important.

Lack of an age-matched, healthy control group was the main limitation of this study. In addition, some of the patients in the study had attempted medical therapy previously. The present authors believe that choosing a group of patients with initial diagnosis of POF may be a better approach for further studies.

Conclusion

It is apparent that BMD is commonly less in women with POF than normal healthy women. Therefore, measurement of BMD is warranted. At this time, it is not clear how often the tests should be carried out to evaluate BMD. Further prospective studies are required to establish guidelines. However, it seems reasonable to monitor women with POF yearly for the presence of any endocrine dysfunction and to assess BMD at periodic intervals.

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The influence of mifepristone to caspase 3 expression in adenomyosis

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Summary

Objective: To discuss the influence of mifepristone to caspase 3 expression in adenomyosis tissue. *Materials and Methods:* Sixty patients were equally divided into four groups. Groups 1,2, and 3 were treated with 5, 10, and 15 mg mifepristone, respectively and group 4 was treated with placebo. The expression of caspase 3 was examined by immunohistochemical method in both eutopic and ectopic endometria of the 40 cases. *Results:* Compared with placebo group, the expression of caspase 3 in both eutopic endometrium and ectopic endometrium in the three treatment groups was significantly increased. There was no difference in the expression of caspase 3 in both eutopic and ectopic endometria between the ten and 25 mg treatment groups, while both the ten and 25 mg treatment groups had a higher expression intensity of caspase 3 in both eutopic and ectopic endometria, compared with the five mg treatment group ($p < 0.01$). *Conclusion:* Mifepristone can increase the expression of caspase 3 in both eutopic and ectopic endometria and initiate cell apoptosis in both eutopic and ectopic endometria. Therefore mifepristone can effectively inhibit the emergence and development of adenomyosis.

Key words: Adenomyosis; Apoptosis; Caspase3; Mifepristone.

Introduction

Adenomyosis (AM) is a disease caused by the endometrum penetrating into the myometrium and growing into ectopic glandular tissue. It is often dispersed in the myometrium and is a common gynecological disease prevalent in women aged 40-50 years [1]. It is a medical condition characterized by volume increase, prolonged menstrual period, and dysmenorrhea which is sexually aggravated [2]. The incidence rate shows an increasing trend, its cause of dysmenorrhea, menorrhagia, and anemia, leading to infertility causes a great deal of pain and mental burden to patients and families. Severe dysmenorrhea especially affects the patient's life and work quality. The incidence of AM has gradually increased over many years and there is a trend of younger women reported at home and abroad.

There is still no ideal treatment for AM. Clinical treatment options range from use of drug therapy, surgery, and uterine artery embolization (UAE). Treatment of AM uterine artery embolization is a novel approach that not only can ease the symptoms of dysmenorrhea, be able to retain the uterus, and have very clear short-term effects, but it still requires further in-depth evaluation regarding the recurrence rate, lesion vascular recanalization, and its influence on ovarian and reproductive function with longer-term studies [3, 4].

Clinically, surgery is still the main treatment, and generally includes hysterectomy, but patients that become infertile can also often lead to endocrine disorders, and even reduce the quality of life after hysterectomy. Therefore, many of the younger patients often cannot accept these side-effects. Drug treatment in AM can relieve the symptoms of dysmenorrhea, but cannot completely cure, the side-effects of the drug, and AM is still very easy to relapse after discontinuation [5]. There is a certain lack of treatment options and this is due to the pathogenesis of AM which remains unclear.

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. Biochemical events lead to programmed cell degeneration and necrosis of the process [6]. Spontaneous apoptosis of endometrial cells is a key factor for metrial tissue to maintain its normal structure and function, and ectopic endometrial cells to grow and continue to survive outside the uterine cavity are particularity related to the changes in cell apoptosis and proliferation. Recent studies have found that there were significant differences between AM endometrial cells and normal endometrial apoptosis rates. Abnormal apoptosis is an important reason for the ectopic implantation and the growth of endometrial cells. Abnormal apoptosis may be a key cause for the occurrence and development of uterine gland muscle disease [7, 8].

Caspase family is widely present in the cells mainly in the form of the zymogen. As an important process of the cell death, once the cutting of caspase is activated, cell death will inevitably occur [9]. Fourteen or more types of the caspase family have been identified; caspase 3 is the most important member of this family. Most of the factors that trigger apoptosis eventually require signal transduction pathway mediated by caspase 3 leading to apoptosis [10]. It is not yet clear about the expression level and its clinical significance of caspase in endometriosis (EM) and AM endometrial carcinoma. In the study of the treatment of en-

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dometriosis using BAY 11-7085 inhibitor of nuclear factor called, Nasu *et al.* [11] found that BAY 11-7085 can activate apoptosis caspase common pathway of caspase 8, 9, and finally activates factor caspase 3, to accelerate the eutopic of apoptosis and ectopic endometrial cells, achieving the purpose of the treatment of endometriosis. This shows that caspase 8, 9 and caspase-3 activity decreased in AM eutopic and ectopic endometrial cells. Kim *et al.* [12] found that the expression level of caspase 3 in endometriosis group was significantly lower than the normal control group and the ectopic endometrial activity than the corresponding lower eutopic endometrium. All these may indicate that in utero film endometriosis endometrium low expression of caspase 3 results in the significantly reduced apoptosis rate in the EMs ectopic endometrial cell apoptosis in the process of EM occurred. The reduced apoptosis rate undermines the balance of cell proliferation and apoptosis. Cell death is less than the proliferation, cell accumulation, and prolonged survival, and leads to the implantation and growth of these cells, which promoted EMs. Therefore, caspase 3 plays an important role in the development of in Ems and may be a new target for the treatment of EMs [13].

Mifepristone, synthetic 19-demethyl-testosterone derivative, has anti-progesterone and anti corticosteroids activity. Previous studies confirm that the mifepristone block progesterone through binding to its receptor [14]. It is the anti-progesterone drugs played on the level of the receptor, can suppress ovarian function, and induce amenorrhea, and make ectopic endometrial atrophy [15]. Reinsch *et al.* [16] reported that after mifepristone (25 mg/d) three months, uterine artery blood flow of patients is reduced by up to 40%, significantly reduced uterine volume, and elevated resistance index. Similar to these results Zhou *et al.* [17] reported through animal tests, that mifepristone is not only able to suppress the occurrence of mice AM, while narrows mice AM lesion, but also relieves symptoms. These results in humans are consistent in the treatment of AM by using mifepristone [18]. Clinical trials confirmed that mifepristone can maintain the patient's blood E2 level in early or mid follicular phase. Furthermore, mifepristone, is more economic, easier to accept, and does not cause bone loss. Therefore, it opens up a new field for the treatment of AM. In recent years, scholars studied the relationship between AM and apotosis. Some studies have shown that mifepristone may promote apoptosis by acting directly on endometrial cells [19]. Mifepristone used in the clinical treatment of AM has a certain effect, [20] but its mechanism of action and therapeutic dose has yet to be further understood.

The study by detecting the expression of cysteine-aspartate-specific protease (caspase 3) in the AM, make the role of caspase 3 clear in the regulation of apoptosis in AM development, and explore influences of the caspase 3 expression on eutopic and ectopic endometrial cells by using different doses of mifepristone in patients with AM and determine the mechanism of mifepristone treatment on AM.

Table 1. — *The ectopic Caspase3 expression level.*

Groups	n	Caspase 3 expression level –	+	++	+++	Positive rate
Control	15	13	1	1	0	13.3%
5 mg Group A	15	4	6	3	2	73.3%
10 mg Group B	15	1	3	5	6	93.3%
25 mg Group C	15	1	2	6	6	93.3%

A, B, and C with the control group compared to the expression intensity of $p < 0.05$; positive rate of $p < 0.01$.
Group B compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C and group B ratio of expression intensity $p > 0.05$; positive rate $p > 0.05$.

Table 2. — *The ectopic endometrial Caspase3 expression level.*

Groups	n	Caspase 3 expression level –	+	++	+++	Positive rate
Control	15	12	2	1	0	20%
5 mg Group A	15	3	6	4	2	80%
10 mg Group B	15	1	4	5	5	93.3%
25 mg Group C	15	1	2	5	7	93.3%

A, B, and C with the control group compared to the expression intensity of $p < 0.05$; positive rate of $p < 0.05$.
Group B compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C and group B ratio of expression intensity $p > 0.05$; positive rate $p > 0.05$.

Material and Methods

The study target

Patients who accepted hysterectomy in the present hospital were selected from October 2008 to October 2010 .The same consented to participate in this research project and signed the consent form and were a total of 60 patients. The patients were randomly divided into four groups. Group A took no medicine for preoperative medication treatment, group B took mifepristone at dose of five mg qd for three months before surgery, group C took mifepristone at dose of ten mg qd for three months before surgery, and group D took mifepristone at a dose of 25 mg qd for three months before surgery. Mifepristone was dispensed to patients by the research group and methods and precautions were explained. The four groups of patients underwent hysterectomy after treatment and observation. Desired eutopic endometrium and myometrium ectopic endometrium were fixed by paraformaldehyde and then embedded with paraffin. The specimens were stained and the expression levels of caspase 3 by immunohistochemistry. Patients had an age of 41 to 49 years, with an average age of 44.54, were women who had given birth, menstrual regulations, with no liver and kidney diseases, no blood diseases, and endocrine history. The age of the study group had no significant difference ($p > 0.05$).

All tissues were paraformaldehyde-fixed, paraffin-embedded, in continuous four-μm thick slices. Immunohistochemistry streptomycin avidin-peroxidase method (SP) was adopted and carried out in the experiments according to the kit instructions.

Observation and judgment of the results.

Caspase 3 expressed in the cytoplasm of epithelial cells and the positive staining cells was the brown granules in the cytoplasm, however, there is no colored nucleus (Figures 1 and 2). Observed

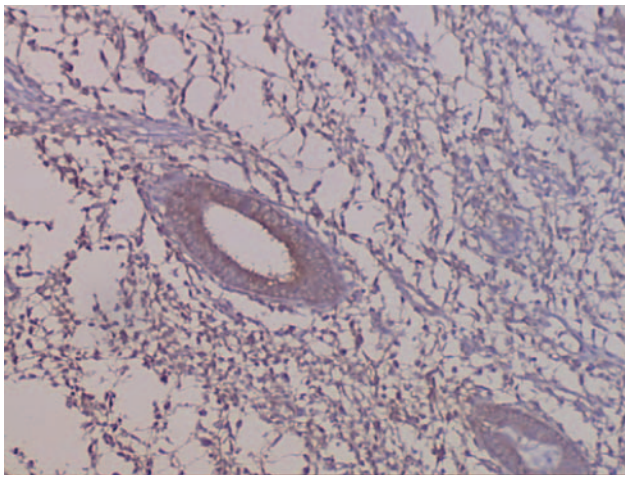

Figure 1. — The ectopic endometrial caspase3 expression, visible brown granules in the cytoplasm of positive cells (×100).

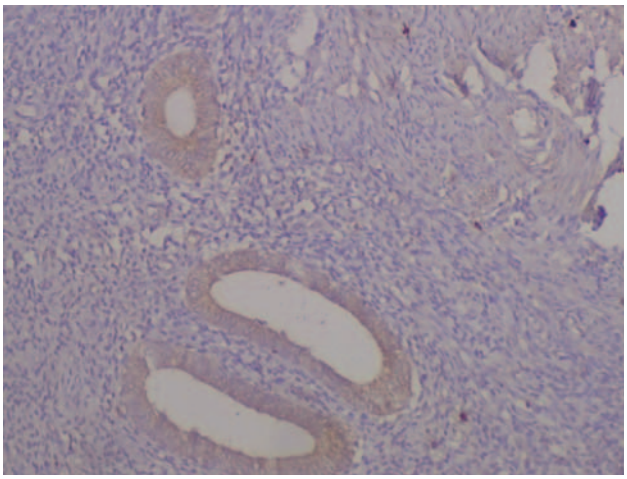

Figure 2. — The ectopic endometrial caspase3 expression, visible brown granules in the cytoplasm of positive cells (×100).

at ten fields of view under a high power microscope and counts were divided into four grades according to the proportion of positive cells: (1) +: positive cells < 25%, (2) ++: positive cells were 25% to 50%; (3) +++: the positive cells > 50% ; (4) cell-brown or light brown is consistent with the background of the negative.

Experimental data were processed using statistical software SPSS 17.0 for Windows. A $p = 0.05$ level of inspection and a $p < 0.05$ of differences were considered significant. Chi-square (X^2) test between the positive rate of statistical methods and expression of strength between the rank-sum test were used for comparison.

Results

The specimens of uterine myometrium eutopic endometria from the four groups of patients were examined immunohistochemically through anti-human caspase 3 antibody. The results showed that there were visible caspase 3 expressions in the two cases samples in the control group, 11 cases in five mg treatment group, and 14 cases in ten mg and 25 mg treatment groups. Compared with unmedicated control group, caspase 3 expression intensity in eutopic endometrium of all the three mifepristone treatment groups were significantly increased. In the two treatment groups of ten mg and 25 mg, the positive expression rate of caspase 3 in ectopic endometrium showed no significant difference. Compared with five mg treatment group, the eutopic positive expression rates of endometrial caspase 3 was significantly different in both. The intensity of expression of each group is shown in Table 1.

The specimens of uterine myometrium ectopic endometria from the four groups of patients were examined immunohistochemically through anti-human caspase 3 antibody. The results showed that there was a visible caspase 3 expression in the three cases samples with the control group, 12 cases in five mg treatment group, 14 cases in ten mg and 25 mg treatment groups. Compared with unmedicated control group, caspase 3 expression intensity in ectopic endometrium of all the three mifepristone treatment groups was significantly increased. In two treatment groups of ten mg and 25 mg, the positive expression rate of caspase 3 in ectopic endometrium showed no significant difference. Compared with five mg treatment group, the eutopic positive expression rates of endometrial caspase 3 was significantly different in both. The intensity of expression of each group is shown in Table 2.

Discussion

This study explores the influence of varying doses of mifepristone in patients with AM eutopic and ectopic endometrial by detecting AM eutopic endometrium and ectopic endometrium cysteine-aspartate-specific proteases (caspase 3) expression level.

Confirmed by this experiment, three mifepristone treatment group compared with the unmedicated control group, eutopic endometrium and ectopic endometrium of caspase 3 expression intensity of both $p < 0.05$, the difference was statistically significant. All these results indicate that long-term use of mifepristone does enhance caspase 3 expression level.

Previous studies have confirmed that the increased expression of caspase 3 can promote apoptosis, and concluded that mifepristone can achieve a therapeutic effect by urging apoptosis of eutopic and ectopic endometrial cells. Ferrero *et al.* showed that mifepristone may directly act on endometrial cells and promote apoptosis [19]. It has been widely confirmed that during mifepristone-treated adenomyosis patients, one mechanism of dysmenorrhea alleviation is that mRNA expression of caspase-3 was

significantly enhanced to promote apoptosis of endometrial cells, which play a role in the treatment, after the drug intervened on glandular epithelial cell proliferative endometrium of patients with adenomyosis.

The results in this study further identified the treatment effect of mifepristone on adenomyosis and support the studies from the scholars at home and abroad, and may provide a theoretical data for future studying adenomyosis treatment from apoptosis.

Over long periods, a large number of oral mifepristone may affect the patient's liver and kidney function. Severe cases may lead to liver, kidney, and adrenal failure. Therefore it is particularly important to find a minimum effective dose. Most frequently-used clinical application dose of mifepristone is 25 mg qd. This study carried out three different doses of mifepristone treatment groups to detect the caspase 3 expression level. There was no significant difference in the positive expression intensity of caspase 3 between 10 mg and 25 mg treatment groups, but compared to the five mg treatment, positive expression rate of caspase 3 in the eutopic and ectopic endometria was significantly increased in both 10 mg and 25 mg treatment groups. This provides a new way of thinking for exploring the optimal dose of mifepristone treatment for AM.

Due to the small samples in this study and the fact that only caspase 3 expression was studied in the factors affecting apoptosis, may have influenced the accuracy of the final results. In the next study, the number of sample cases and expressions of other apoptotic factors detected in adenomyosis endometrium should be increased, and a better method of treatment of adenomyosis and drug dose will be found to relieve the suffering of patients.

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Homeopathy for infertility treatment: a case series

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Summary

Homeopathy has been used in the past for treating a broad aspect of diseases. In gynecology, its use remains limited. Taking under consideration its clinical aspects, the authors attempted to use it for treating female sub fertility problems. With this study, the authors present five cases of female infertility treated successfully with the use of homeopathic treatment in a large obstetrics-gynecology Hospital in Athens.

Key words: Homeopathy; Infertility.

Introduction

Fertility issues are very common nowadays as almost one out of seven couples of reproductive age encounter infertility during their reproductive years [1]. Infertility is defined as the inability to achieve a pregnancy after one year of regular unprotected intercourse [2-5].

Many advances have been recorded the last decades in assisted reproduction techniques (ART) resulting in much better results, not only in live birth rates but also in womens' overall health. Despite great achievements and advances in ART techniques, still overall success rates need to be improved. Moreover, financial costs and emotional distress, for both couples and medical personnel, are related with treatment procedures [6-9]

For all these reasons couples tend, in addition to conventional ART therapies, to use methods of complementary or alternative medicine (CAM), in an attempt to achieve pregnancy [1].

Therefore, a number of treatments (such as acupuncture, traditional Chinese herbal use, etc.) [1, 10, 11] have been proposed for improving ART outcomes and some of them have already proven effective. One of the established CAM methods is homeopathy. Homeopathy use has been used in the past for treating several different diseases and, among them, infertility in both animals and human [12-14].

With the present study, the authors report a series of five cases of infertility of different etiology treated with the use of homeopathic medication.

Materials and Methods

All patients have been followed in the Sixth Obstetrics- Gynecologic Department of "Helena Venizelos" Hospital of Athens. Patients presented in this study are part of a larger cohort of patients treated for either male, female (due to ovulation disorders or tubal factor infertility) or unexplained infertility.

Table 1 presents demographic characteristics and gynecological history of the patients as well as patients' previous treatments.Table 2 presents patients' homeopathic regimen used for treatment and clinical outcome. All patients were treated with individualized medical treatment diagnosed, as in every homeopathic case, on the global level.

Discussion

To the authors' knowledge, this is one of the first studies reporting homeopathic use for fertility treatment.

Based on homeopathic principles, infertility can be considered as a disruption of the complex systems of networks that ensures body's balance and good health. Such a loss, generates an abnormal pathological trend: infertility. [15] The reason for this, according to homeopathy, is the derangement of body's ability to self-regulate.

Homeopathy tends to re-establish the mind-body entity. This is achieved by individualized homeopathic therapy [16].

In the past, proven positive outcomes in fertility have been reported for acupuncture and herbal treatments [1, 10, 11].

These have prompted the authors' interest in the possible usefulness of homeopathy for treating female factor infertility.

The possible explanations for that are not clearly defined. Homeopathy may have a positive effect on implantation or on the general well-being of the patients which in turn may increase fertility. Some might say that homeopathy may exert its positive effect through the, previously reported, "placebo- effect".

The present results show the positive effect different homeopathic treatment exert in infertility treatment. The various homeopathic drugs used suggest different mechanisms of action, as well as a general well-being of the pa-

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Table 1 – *Patients' demographic characteristics – gynecological history*

Names (patients' initials)	Date	Age	Type of Infertility	Infertility pathophysiology
TK	1/2011	41	Secondary	Unexplained
NR	4/2010	29	Primary	Anovulation
KP	11/2010	38	Secondary	Elevated FSH- secondary anovulation
GS	4/2010	32	Primary	PCOS
SP	1/2011	39	Primary	Endometriosis (Stage II- after laparoscopy)

Table 2 – *Homeopathic treatments and clinical outcome*

Names (patients' initials)	Homeopathic treatment	Therapeutic outcome
TK	SEPIA 1m+6x/60	Pregnancy, 4 months after treatment
NR	MEDORRHINUM 200+ KALI PHOSPHORICUM 6x/40	Pregnancy, 1 month after treatment
KP	CALCAREA CARBONICA 200+6x/60	Pregnancy, 1.5 month after treatment
GS	IGNATIA 10M+6x/60	Pregnancy, 10 months after treatment
SP	CACTUS 30ch+6x/100	Pregnancy, 4 months after treatment

tients achieved. That may be due to stress – relief or any other positive indirect action of the drugs used.

Moreover, the different time needed for achieving a positive therapeutic result, may show different therapeutic action of the different drugs and/or different reaction of women treated.

All these should be proved with large scale, randomized controlled trials examining homeopathy's use for fertility. Stratification for each cause of fertility, patients' age, previous treatment etc. will help determining with statistic significance homeopathy's effect on fertility. Case series, as in the present study, may present a trend, but cannot definitely prove a relationship between a drug and a disease. Despite this, the present study is helpful in that it may lead to the conduction of randomized controlled trials. The beneficial effects presented in this study may be attributed to homeopathic drug use but its limitations (case series) are such that larger, higher quality studies are required in order to establish the causative relationship between infertility treatment and homeopathy.

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Correlations of abnormal ultrasound audio-visual images of ovarian cortex surface and pelvic adhesion in infertile patients

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Summary

Objective: The aim of this study was to evaluate the related factors between abnormal ultrasonic appearance of ovarian and pelvic adhesion in infertile women. *Materials and Methods:* Forty-eight cases were examined with transvaginal ultrasonography (TVUS) if there was pelvic adhesion before surgery (experiment group), and the surgical group was used as control. The specificity of pelvic adhesion was evaluated. *Results:* Thirty-nine cases were abnormal in experiment group and 38 cases were confirmed with surgery, while one case was normal. Nine cases were normal in study group and six cases were confirmed with surgery, while three cases were abnormal. There were 91.7% (44/48) in coincidence rate and 97.4% (38/39) in positive predictive value. *Conclusion:* Infertility in women with pelvic adhesion with abnormal ovarian appearance, may be examined specifically with TVUS

Key words: Laparoscopy; Infertility; Pelvic adhesion.

Introduction

Pelvic adhesion is a common gynecologic disease, often caused by infection, endometriosis, surgical trauma, foreign body reaction, organic ischemia, etc. It could lead to intestinal obstruction, infertility in women, failure in fertility reconstruction surgery, discomfort sexual life, chronic pelvic pain, and increase of secondary operation rate [1]. Infertility is a serious reproductive health problem, with different incidence in different countries and regions [2]. It was pointed out by "2004 birth guide" [3] that tubal is a high-risk factor leading to infertility, accounting for 30%~40% of infertile etiologies. Pelvic adhesion destroys pelvic anatomical structure and limited tubal function, leading to infertility. Pelvic adhesion is usually diagnosed through the laparoscopic surgery, etc. [4], with only very few cases through single ultrasound diagnosis, such as hydrosalpinx and empyema, tubo-ovarian abscess, and inflammatory masses, etc. If pelvic adhesion is diagnosed preoperatively, the pathogeny of infertility could be more accurately assessed and its therapeutic regimen could be made in time, such as assisted reproductive technology, etc. However at present, there is no report revealing clear evidences of pelvic adhesion preoperatively. Then, could a non-invasive method be found to predict the existence of pelvic adhesion?

There are many causes of pelvic adhesion, mainly including damage of peritoneal and serosa of abdominal organs basin, ischemia, and local inflammatory reaction caused by infection, etc. This could be caused by mechanical injury, physical injury, infection, foreign bodies, and allergic reaction, etc. [5-7]. In addition, pelvic adhesion could also be caused by diseases and treatments themselves, such as peritonitis, pelvic inflammation or peritoneal dialysis, etc [8]. From the occurrence mechanism of adhesion, the exudate rich in fibrin could raise macrophages and fibroblasts to inflammation parts; fibrinolytic activity could be declined, stopping the removal of fibrin deposits; hyperplasia of vascular granulation tissue, along with the deposition of collagen and elastin in the fibronectin glycans grid structure in the area of injury, could cause excessive fiber formation, beyond the clear ability of macrophages, thus adhesion could be formatted [9]. Adhesion is constituted by excessive fibrosis caused by accumulation of granulation tissues and fibronectins. Excessive fibrosis tissues could show strong echo under the ultrasonic. When the inflammation of abdominal organs basin spreads around the ovary, the local adhesive lesion attaches to the surface of the ovarian cortex. Compared with the low-echo ovarian acoustic image ovary, could it show an abnormal ultrasound image? What is the correlation of this abnormal ultrasound image along with the occurrence of pelvic adhesion? Could it be used as diagnostic evidences of pelvic adhesion?

In the field of gynecology and obstetrics, ultrasound examination has become one of the essential methods in clinical diagnosis. Compared with abdominal ultrasound, transvaginal ultrasound (TVUS) could not be disturbed by fat, abdomen scar or flatulence, with a high probe frequency and a clear image. Owing to the small volume and changeable position, clear ovarian boundary cannot be easily assessed by abdominal ultrasound. Vaginal probe could be rotated 360 degrees intrapelvically, expanding the vision in order to seek a varied ovarian position. TVUS observation of the ovary could

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easily determine its position and show ovarian outline. In the early project funded by the science and technology commissions in Lianyungang City "study on tubal function examined by pelvic imaging techniques through transvaginal B ultrasonic" (invasive operation), there was a kind of ultrasound images closely related to pelvic adhesion. This image could be clearly shown not only in the imaging techniques to pelvic affusion, but also in regular vaginal ultrasound examination. Hence could this kind of ultrasound images be non-invasive evidences of pelvic adhesion?

As the understanding of the pathological mechanisms behind pelvic adhesion deepens and technology advances, vaginal ultrasound has been widely used for ovary examination because of its advantages. This study aimed to explore the correlation of ultrasound audio-visual abnormality on the surface of ovarian cortex and pelvic adhesion in infertile patients, by comparing the results of ovarian audio-visual images with the results of surgery and evaluating the positive predictive value of pelvic adhesion.

Materials and Methods

Objects

There were 48 cases of infertility patients received laparoscopic or laparotomy in Lianyungang Maternity and Child Healthcare Hospital of Jiangsu from December 2006 to May 2008. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Lianyungang Maternity and Child Healthcare Hospital of Jiangsu. Written informed consent was also obtained from all participants.

Experiment group

The preoperative selected patients were given ALOKA1000 type ultrasonic inspection, with 5.0 MHz of ultrasound frequency vaginal probe. Pelvic scanning had the aim to acquire ultrasound audio-visual images, in which ovarian cortex were described and preliminarily diagnosed by ultrasound imaging on the basis of routine reports. Patients receiving ultrasonic inspection comprised the experimental groups (TVUS groups) and those receiving intraoperative diagnosis comprised the control groups (surgical groups). The surgical groups were subject to routine operation on the abdominal cavity. The anatomical structure was described and the interrelations among the omentum majus, bowel, uterus, ovary, oviduct, and rectouterine fossa were detected. The density degree, the range of pelvic adhesion, the status of Douglas pouch, the adhesion of bilaterla ovaries and oviducts to the surrounding tissues, and the presence or absence of oviduct atresia were determined.

Decision criteria

TVUS groups: Normal: The outline border of ovarian cortex was clear or not, without unusual echoes on the surface. Abnormal: the outline border of ovarian cortex was clear or not, with scattered or dense enhanced light point or facula on the cortical surface, with or without pelvic effusion. Surgical groups: Normal: the morphology and anatomy of adnexa uteri were normal, without adhesion. Abnormal: there were different degrees of adhesions in abdominopelvic cavity.

Evaluation indexes

The specificity of experiment groups was evaluated by contrasting results of experiment groups to control surgical groups considered as standard.

Results

Common condition

There were 48 cases of infertility patients, aged 21-45 years, 15 cases of primary infertility, and 33 cases of secondary infertility. Among the 15 cases, there were five cases of tubal identical operation, four cases of polycystic ovary syndrome, two cases of mediastinum uterine, four cases of ovarian cyst, and 33 case of tubal factors.

Results of diagnosed pelvic adhesion in two groups

TVUS groups: among 48 cases of patients, there were 39 cases whose outline border of ovarian cortex were clear or not, with scattered or dense enhanced light points or facula on the cortical surface, with or without pelvic effusion and nine cases were normal.

Surgical groups: among 48 cases of patients, there were 41 cases with different degrees of adhesions in abdominopelvic cavity and seven cases were normal.

Contrast between two Groups: among 39 cases of abnormal ultrasonic inspection, there were 38 cases of adhesion and one normal case confirmed by surgery. Among nine cases of normal ultrasonic inspection, there were six normal cases and three cases with adhesion, as confirmed by surgery. The total coincidence was 91.7% (44/48) and the positive predictive value was 97.4% (38/39).

Discussion

Evaluating tubal normality plays a vital role in the diagnosis and treatment of infertility. Part cases of the infertility patients were diagnosed with a normal oviduct, but confirmed to have different degrees of adhesions in pelvic cavity by following surgeries. These neglected problems could lead to abnormal tubal function and infertility. At present, the methods utilized include tubal liquid instillations, X-ray hysterosalpinography (HSG), etc. Such techniques are easily performed and the unobstruction of oviduct could be preliminarily determined. However, in some cases in which the oviduct is diagnosed unobstructed, while later pelvic adhesion and abnormal tubal function are found in the subsequent surgical results. The anatomical features of female pelvis show interconnection with the external environment through oviduct and uterus, and there are usually three infection paths. One path is an uplink infection along with reproductive organs mucosa, such as gonorrhea infecting cervical mucosa, endometrium, and tubal mucosa. Another path is pathogenic bacteria extending to oviduct along with the portio supravaginalis and cervical retroperitoneal lymphatic system. Initial infected area began with the surface of the oviduct and ovary and the actual infection occurs after miscarriage, in puerperium or when placing intrauterine device. The third path is disseminated through the blood. The focus of infection could infect peritoneum through circulation and then infect oviduct, resulting in tuberculous salpingitis.

Therefore, the female pelvic infection could initially spread to tubal and ovarian surroundings. Meanwhile, the constitution of adhesion could be observed: it was formed by excessive fibrosis as a result of the accumulation of granulation tissues and fibronectins. Owing to the low-echo in ovarian normal ultrasonogram and the hyperecho in normal ultrasonogram of fibrotic tissues, the contrast could be clearly displayed under vagina ultrasound when these fibrotic tissues adhere to the ovarian surface. In this article, 48 cases of infertile patients were compared and a prediction was made whether there would be adhesions in the pelvic cavity through TVUS. As a result, there were 39 TVUS cases with abnormality revealing adhesion; of these, there were 38 cases with confirmed adhesion and one case as normal through surgery. The positive predictive value was 97.4%. It could be confirmed that abnormal images of ovarian surface could be shown by TVUS in order to predict the existence of pelvic adhesion (with scattered or dense enhanced light point or facula on the cortical surface). If corresponding therapeutic regimens are timely made, these patients could avoid delaying the best pregnancy time, which would play an auxiliary diagnosis function to some gynecological pelvic diseases, such as chronic pelvic pain at the same time.

The pelvic structure can be altered by pelvic adhesion. It is one of the causes of infertility due to chronic pelvic inflammation which interferes with the sweeping of the oocyte function of oviduct and the transportation function of fertilized oocytes. The normal anatomical structure of pelvic cavity and unobstruction of oviduct are recovered through adhesiolysis and then function of oviduct is restored. There are many reports regarding the correlation of surgery and pregnancy, part of which have proved the validity of laparoscopy in this field [10-12]; at the same time, Yaron *et al.* reported that 96% appeared with adhesion again after laparoscopic adhesiolysis; only 50% reduced the adhesion scores. While there were still 67% adhesions that reformed in the area of adhesiolysis by secondary laparoscopy [13], other researches proposed that both oviduct lesions and pelvic adhesion had no influence on postoperative pregnancy [12, 14]. Therefore, the pregnancy probability needs to be reasonably evaluated in the treatment of infertility and pelvic adhesion. The problem of fertility needs to be solved through minimally invasive surgery or assisted reproductive technology with a reasonable therapeutic regimen.

There were various classification methods to lesion degrees of pelvic adhesion, such as the scoring methods of oviduct lesions degree combined with organic adhesion degree put forward by Canis *et al.* [11], and the classification standard proposed by American Fertility Society (AFS) [15], but not all of the abnormal images of ovarian surface do show pelvic adhesions by TVUS. This depends on the degrees and positions of adhesion lesions. Firstly, slight adhesion could not form enough fibrosis tissues to show abnormal audio-visual images; secondly, the position of adhesion has nothing to do with ovary. Furthermore, more accurate evaluated data can be acquired through comparing the correlation of different degrees of adhesion and abnormal images of ovarian surface according to the classification standard.

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Does proteinura in preeclampsia have enough value to predict pregnancy outcome?

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Summary

Objective: Preeclampsia is defined by the new onset of elevated blood pressure and protienuria after 20 weeks of gestation. Protienuria is one of the essential criteria for the clinical definition of preeclampsia. The authors investigated the predictive value of proteinuria in the outcome of pregnancies with preeclampsia. *Materials and Methods:* In this retrospective cohort study, they entered all pregnant women who were admitted with diagnosis of preeclampsia in Yahyanejad Hospital from 1998 to 2008. Patients' data such as age, gestational age, level of 24-hour urine protein, liver enzyme, blood urea nitrogen (BUN), creatinine, and other laboratory test. Also, prenatal and maternal outcome were studied. The data analyzed and compare with each other. *Results:* Out of 289 patients, 5.9% (17) women had placental abruption, 13.1 % (28) patients had intrauterine growth retardation (IUGR), 32.2% (96) had respiratory distress, and 26.6% (77) of the patients' infants were transferred to neonatal intensive care unit (NICU). Although the present study showed proteinurea cannot be a sufficient predictor for adverse consequences of preeclampsia, however, the incidence of pregnancy adverse effects increased in the patients with elevated 24-hour proteinuria. *Conclusion:* The authors concluded that proteinuria in patients with preeclampsia is associated with adverse outcome in pregnancy, although it is not an adequate predictor.

Key words: Preeclampsia; Proteinuria; Pregnancy outcome; Hypertention.

Introduction

Among hypertensive disorders in pregnancy, preeclampsia is known as a major cause of maternal and fetal mortality. Preeclampsia is referred to hypertension with proteinuria occurring after 20 th week of pregnancy in a woman with a normal blood pressure [1]. Clinical symptoms of preeclampsia usually occur at any time after the second trimester. Preeclampsia occurs in about 3.9 percent of pregnancies worldwide [2] and is defined as a particular pregnancy syndrome which can generally affect the whole body systems. The disease occurs in multiple organs such as kidneys, liver, and also brain and causes constant adverse effects [3]. The combination of proteinuria and hypertension is associated with adverse outcomes of pregnancy such as low neonatal consciousness at birth, intrauterine fetal death (IUFD), low birth weight (LBW), intrauterine growth restriction (IUGR) requiring admittance to neonatal intensive care unit (NICU). [4]. Gestational high blood pressure may be misleading because mild disease could quickly become severe [1]. Proteinuria is considered in the diagnosis of preeclampsia –eclampsia. These symptoms may appeared with a delay. Although, some women may have suffered the seizure before the onset of proteinuria, or mandatory delivery due to preeclampsia, however 17% of patients with eclampsia, did not have proteinuria at the time of the seizure [5].

Proteinuria is an important criterion for preeclampsia and is a specific test to assess severity of the disease and predict the consequences in the women suffering from preeclampsia [6] Proteinuria in preeclampsia occurs following damage to the glomeruli of renal endothelium. Impaired placental blood flow causes less perfusion and hypoxia, and ultimately leads to the release of placental debris and causes a systemic inflammatory response [7-8]. The coherence between proteinuria and adverse fetal outcomes were first considered first by Page and Christanson [9]. Later, other studies showed that the increased protein excretion in women with preeclampsia was associated with fetal and maternal adverse outcomes [10, 11]. However, these initial studies, not only lacked the adequate sample size, but also the numbers which were presented as the cutoff points of urinary protein levels, demonstrated clear discrepancies.

A group of researchers in the United States reported that about 16% out of 3,201 maternal deaths were owing to high blood pressure consequences in pregnancy. It is necessary to say that they reported later more than half of these deaths were preventable [12, 13].

Some investigators have evaluated microalbuminuria as a potential predictive test for preeclampsia. Sensitivity fluctuated from seven to 90% and specificity from 29% to 97%, respectively. It indicated a low clinical predictive value [7]. Newman *et al*. admitted 209 patients with a diagnosis of pre-eclampsia, of which 125 women had proteinuria less than five g/h, 43 women had five to ten g/h, and 41 women had more than ten g/h. No significant dif-

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ferences of the maternal and fetal complications were observed between the three groups [14]. Also, Thangaratinam *et al.* studied the maternal complications in the three-point cut-off of proteinuria at two, five, and ten g. The results defined proteinuria as a poor predictor of maternal and fetal complications in the women with preeclampsia [6]. Although, Gangaram *et al.* in their study on 163 women with hypertension during pregnancy, concluded the consequences are more intense when proteinuria is associated with hypertension [15]. A review article by Lindheimer *et al.* suggested evaluating physiologic kidney function in protein excretion is necessary for proteinuria assessment. Also, cutoff point for abnormal proteinuria was only used to diagnose pre-eclampsia and not as guidance for management of disease [7]. The aim of this study was to identify the predictive value of proteinuria in pregnancy outcome with preeclampsia.

Materials and Methods

In this retrospective cohort study, all patients admitted with the diagnosis of preeclampsia and eclampsia in Yahyanegad Hospital in Babol (north of Iran) from 2000 to 2010, were entered in the study.

Exclusion criteria consisted in patients with kidney disease, those with background of hypertension, and chronic proteinuria.

The definition of preeclampsia according to the National High Blood Pressure Education Program (NHBPEP) Working Group was used [16]. Preeclampsia was defined as systolic pressure 140 mmHg and/or diastolic pressure 90 mmHg on two occasions, at least six hours apart, and proteinuria with a urinary total protein of 300 mg/24 hours in a single specimen occurring for the first time in the second half of pregnancy [17]. All pregnant women with above criteria were included.

Maternal age, gestational age, parity, clinical symptoms (headache, blurred vision, epigastric pain, oliguria), laboratory findings (complete blood count (CBC), platelet, liver function tests, BUN, creatinine (CR), protein 24 hours), maternal complications (pulmonary edema, kidney damage, blood transfusion, liver complications, transfer to the ICU, incidence of caesarean section, maternal mortality), and prenatal complications (preterm labor, placenta abruption, IUGR, fetal death, fetal distress, Apgar scores [16] at five minutes were recorded. Significant difference was considered at $p \leq 0.05$.

Patients were divided into two groups: Group A: patients with gestational hypertension and proteinuria. Group B: patients with gestational hypertension and proteinuria and changes in clinical findings. Outcome of pregnancy in each group was compared with the other groups after entering the data obtained through the patients' files. To calculate, SPSS 16 software was applied. The tests which were used to analyze included ROC curve, T test, and Chi Square.

Results

Over ten years, 570 patients were admitted with preeclampsia in the present hospital. According to inclusion and exclusion criteria, 289 patients remained in the study and were evaluated. Their mean age was 29.46 ± 7.43 years.

In 5.9% of patients (17), the placental abruption occurred. The mean 24-hour urine protein was significantly higher in the patients with placental abruption (p =0.000, Table 1)

IUGR occurred in 13.1% of patients (38). In these patients, mean 24-hour urine protein was higher than the patients who had not IUGR (p =0.000, Table 1). The respiratory distress syndrome (RDS) observed in neonates of 33.2% patients (96) and infants of 26.6% patients (77) were transferred to the NICU. Both of these patients had 24-hour urine protein levels higher than other patients (in both cases p =0.000, Table 1).

Using ROC curve, the cutoff points for 24-hour urine protein was 1,250 mg in RDS (Figure 1), 1,750 mg in the placental abruption (Figure 2), 1,650 mg in IUGR (Figure 3), and for transfer to the NICU 1,350 mg (Figure 4) were calculated. The sensitivity and specificity of three cutoff points for each outcome show to be correlated with the ROC curve in Table 2.

Tables 3-6 show the correlation between 24-hour proteinuria with the number of women with the pregnancy complications.

Mean 24-hour urine protein was significantly greater in patients with complications in the selective cutoff points.

Out of 289 patients, 207 patients had only proteinuria and categorized in group A. Eighty-two patients had other abnormal tests in addition to proteinuria and were categorized in group B. The mean age of patients in group A was 28.61 ± 6.32 years and 31.53 ± 7.27 in group B (p = 0. 329).

Table 1 – *Twnty-four hour urine proteinuria in patients with or without pregnancy outcome.*

p value	n. (%)		
0.00	17 (5.9)	yes	Placental
	272 (94.1)	no	abruption
0.00	38 (13.1)	yes	IUGR
	251 (86.9)	no	
0.00	96 (33.2)	yes	Respiratory
	193 (66.8)	no	distress
0.00	77 (26.6)	yes	Transfer to
	212 (73.4)	no	NICU

Table 2 – *Sensitivity and specificity of calculated cutoff points for 24-hour protein in predicting pregnancy complications.*

Pregnancy complication	Cutoff point	Sensitivity	Specificity	Roc curve	*p* value
Respiratory distress	1,250	91.7	78.4	0.857	0.000
Placental abruption	1,750	94.1	63.7	0.777	0.009
IUGR	1,650	88.9	64.5	0.793	0.000
Transfer to NICU	1,350	98.7	69.8	0.914	0.000

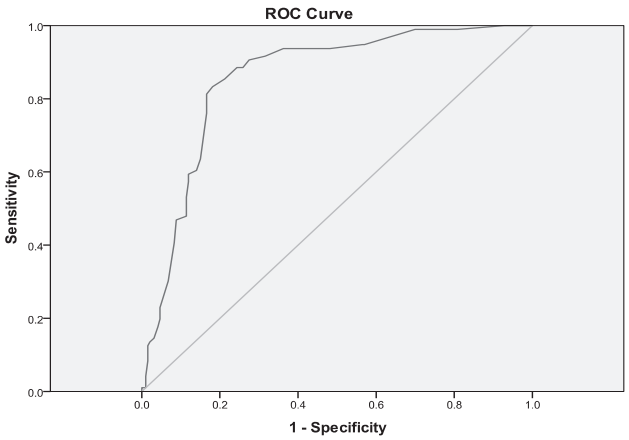

Figure 1 – ROC curve of comparison of 24-hour urine protein or respiratory distress.

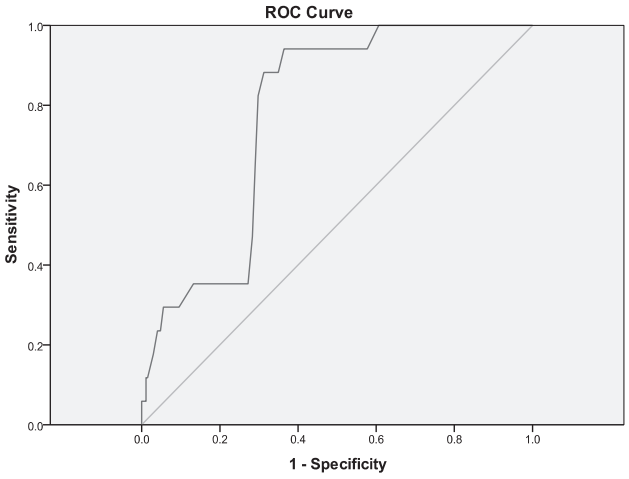

Figure 2 – ROC curve of comparison of urine protein 24 hr or placenta abruption

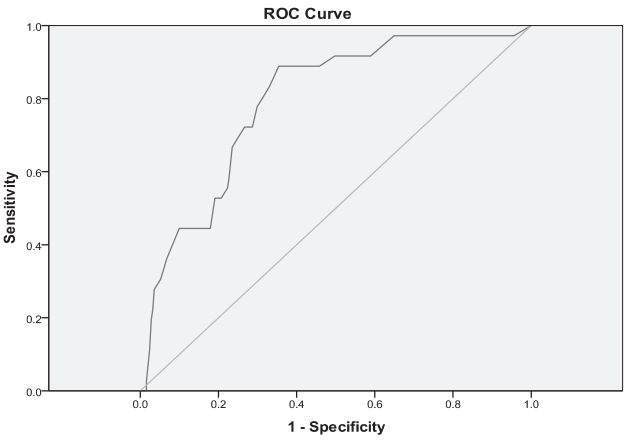

Figure 3 – ROC curve of comparison of 24-hour urine protein intrauterine growth retardation (IUGR).

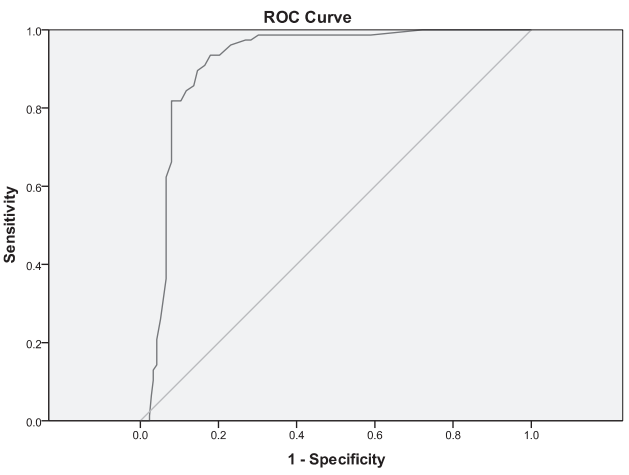

Figure 4 – ROC curve of comparison of 24-hour urine protein neonatal intensive care unit (NICU).

Table 3 – *Relationship of 24-hour protein according to determined cutoff points or respiratory distress.*

		Level of urine protein 24 hr	
		≤ 1,250	> 1,250
		N (%)	N (%)
Respiratory distress	Yes	8 (8.3)	91.7 (88)
	No	132 (68.4)	61 (31.6)

$p = 0.001$

Table 4 – *Relationship of 24-hour protein according to determined cutoff points or abruption placenta-*

		Level of urine protein 24 hr	
		≤ 1650	>1650
		N (%)	N (%)
Abruption placenta	Yes	1(0.6)	16 (94.1)
	No	173 (63.3)	99 (36.4)

$p = 0.000$

Table 5 – *Relationship of protein 24 hr according to determined cutoff points or IUGR*

		Level of urine protein 24 hr	
		≤ 1650	>1650
		N (%)	N (%)
IUGR	Yes	5 (2.9)	162 (97.1)
	No	33 (27.1)	89 (72.9)

$p = 0.000$

Table 6 – *Relationship of 24-hour protein according to determined cutoff points or transfer to NICU.*

		Level of urine protein 24 hr	
		≤ 1350	> 1350
		N (%)	N (%)
Transfer to NICU	Yes	1 (1.3)	76 (98.7)
	No	148 (69.8)	64 (30.2)

$p = 0.001$

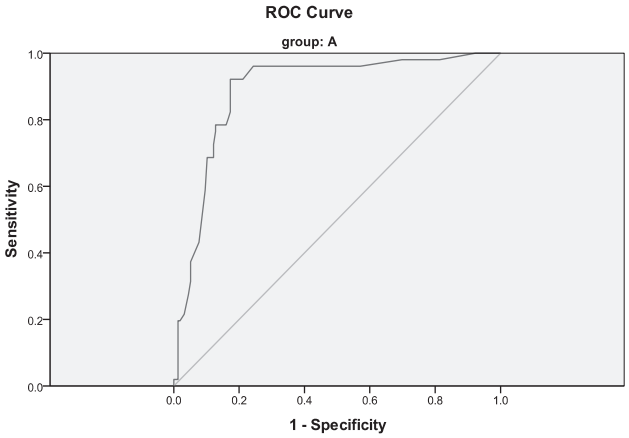

Figure 5 – ROC curve of comparison of 24-hour urine protein or respiratory distress in group A.

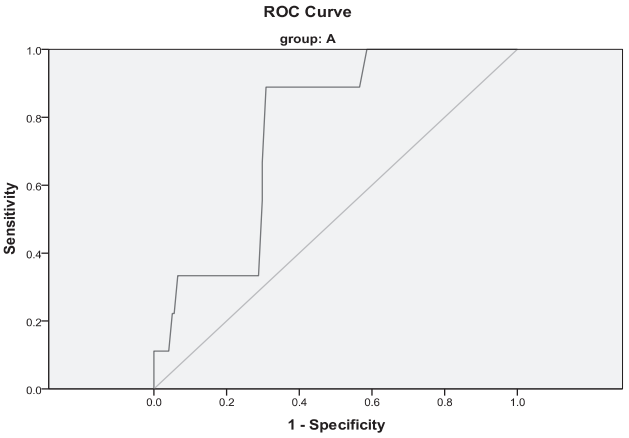

Figure 7 – ROC curve of comparison of 24-hour urine protein or abrubsion placenta in the group A.

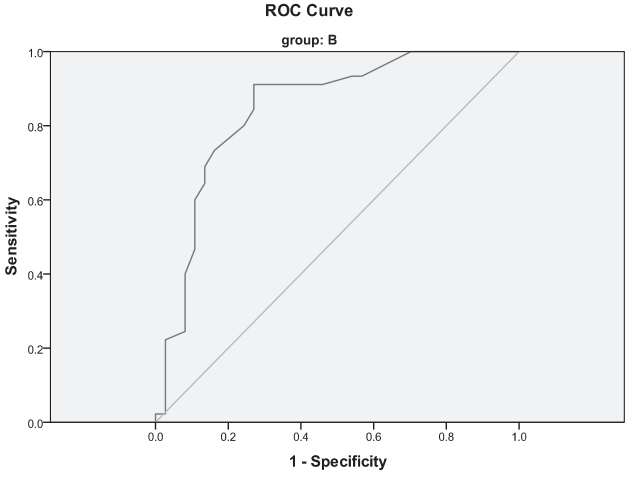

Figure 6 – ROC curve of comparison of 24-hour urine protein or respiratory distress in the group B.

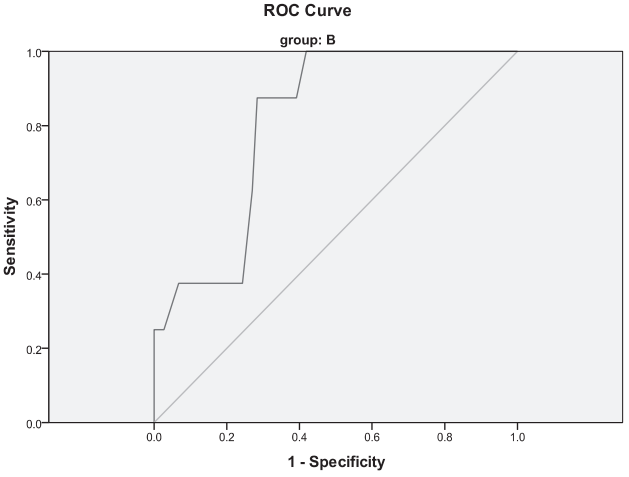

Figure 8 – ROC curve of comparison of 24-hour urine protein or placental abruption in the group B.

Blood transfusion in the whole samples were five patients: two patients in group A and three in group B. Total rate of injected platelets was reported in for patients which were all in group B. The number of IUFD was seven: five belonged to group A and two to group B.

Of 289 deliveries, 97.2% (281) cases were cesarean section and 2.8% (eight) had vaginal delivery: six normal deliveries belonged to group A and two deliveries to group B.

Other complications of groups A and B have shown to compare in Table 7. As can be seen, differences were all significant except in placental abruption.

Using ROC Curve, cutoff point for 24-hour urine protein which occurring in the respiratory distress in group A was 1,550 mg (Figure 5) and 1,150 mg in group B (Figure 6).

The cutoff point of 24-hour urine protein for the placental abruption in group A was 2,050 mg (Figure 7) and 2,150 mg in group B (Figure 8).

In the IUGR, cutoff point for 24-hour urine protein levels in group A and group B were 2,350 mg (Figure 9) and 1,650 mg (Figure 10), respectively.

Also, cutoff points of 24-hour urine protein for infants transferred to NICU in group A and group B were 1,850 mg (Figure 11) and 1,350 mg (Figure 12), respectively.

Sensitivity and specificity of calculated three cutoff points for each outcome and also, areas under the curve are shown according to each group in Table 8.

Discussion

Until now, the disorders of proteinuria in pregnancy have been considered as one of the most interesting challenges in obstetrics. The present study found the maternal adverse effect occurred when mean urinary protein was higher. Brown *et al.* as in the present study showed that maternal

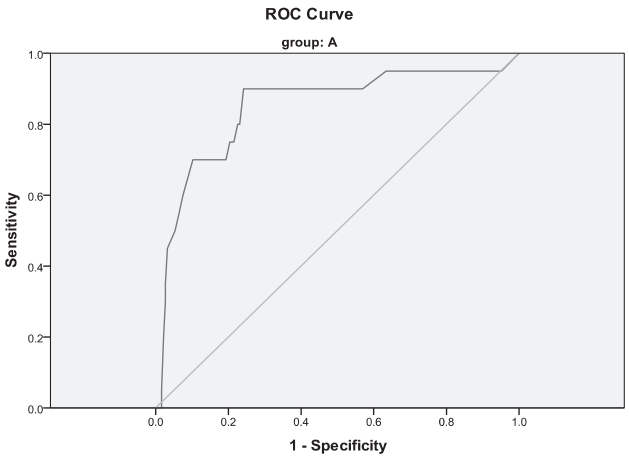

Figure 9 – ROC curve of comparison of 24-hour urine protein or intrauterine growth retardation (IUGR) in group A.

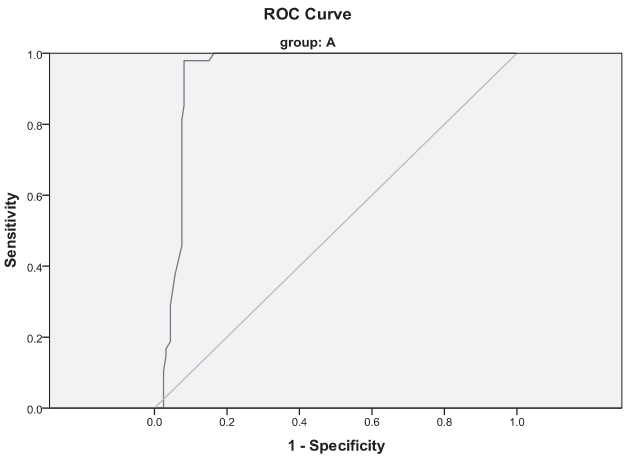

Figure 11 – ROC curve of comparison of 24-hour urine protein or transfer to neonatal intensive care unit (NICU).

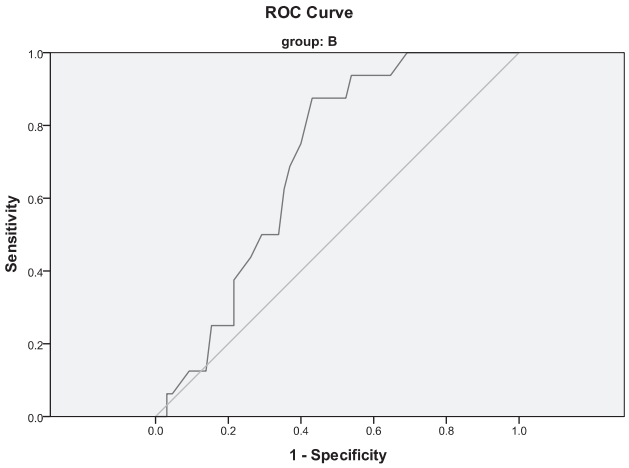

Figure 10 – ROC curve of comparison of 24-hour urine protein or intrauterine growth retardation (IUGR) in group B.

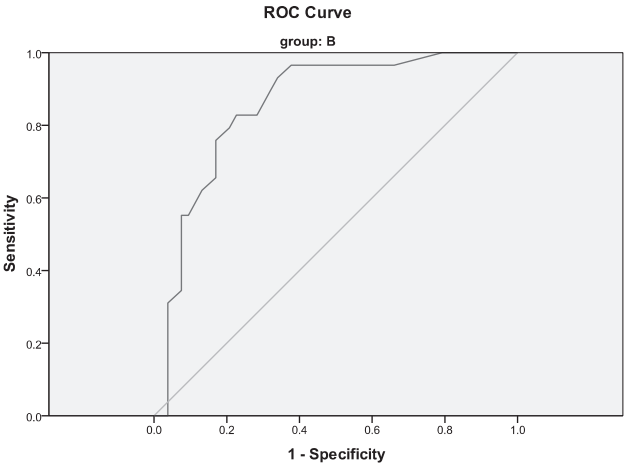

Figure 12 – ROC curve of comparison of 24-hour urine protein or transfer to neonatal intensive care unit (NICU).

proteinuria can be a good predictor [17]. Ferrazzani *et al.*, Gangaram *et al.,* and Chan *et al*. also showed a higher rate of complications when hypertension is associated with proteinuria [15, 18].

There are many conflicting results in the present study. In Thangarayinam, Newman, Nisell, Chua, and Schiff researches proteinuria is not a good predictor for maternal complications [6, 14, 19, 20, 21].

In this study, the authors found the cutoff points of 24-hour urine protein for each adverse outcome: 1,250 mg for RDS, 1,650 mg for IUGR, 1,750 mg for abruption placenta, and 1,350 mg requiring NICU.

Increased of adverse maternal outcomes occurred with a high cutoff point urine protein/creatinine ratio in preeclamptic women at greater than nine g/day, or greater than five g/day in women over 35 years [18]. However, Chua *et al.* studied the women with proteinuria over five grams with no significant increase was observed in the complications when their delivery delayed [20]. Newman *et al.* divided patients into three groups of proteinuria: less than five grams, five to ten grams, and over ten grams No significant differences were seen in complications between the three groups [14].

Thangaratinam *et al.* reviewed 16 articles from years 1951-2007. They evaluated cutoff points ten grams and five grams and increase of protein levels of two grams between the two measurement and determined likelihood ratios (LRs) for positive LR + and negative LR- test results in each of the points, although, they concluded proteinuria is a weak predictive for adverse consequences [6].

In the present authors' further research, they divided the patients into two groups: A and B. Regarding less rate and lower cutoff point of complications in group B vs group A, showed that proteinuria alone cannot be sufficient to predict all adverse consequences.

Table 7 – *Comparison of pregnancy complications in the groups.*

Complication		Group A N (%)	Group B N (%)	*p* value
Abruption placenta	Yes	9 (4.3)	8 (9.8)	0.096
	No	198 (95.7)	74 (90.2)	
IUGR	Yes	20 (9.7)	16 (19.8)	0.029
	No	186 (90.3)	65 (80.2)	
Respiratory distress	Yes	51 (24.6)	45 (54.9)	0.000
	No	156 (75.4)	37 (45.1)	
Transfer to NICU	Yes	48 (23.2)	29 (35.4)	0.039
	No	159 (76.8)	53 (64.6)	
IUFD	Yes	5 (2.4)	2 (2.4)	1.000
	No	202 (97.6)	80 (97.6)	

Table 8 – *Sensitivity and specificity of calculated cutoff points for 24-hour protein in predicting pregnancy outcome.*

Pregnancy complication	Group	Cut of point	Sensitivity	Specificity	Roc curve	*p* value
Respiratory distress	A	1550	96.1	75.6	0.889	0.000
	B	1150	91.1	73	0.846	0.000
Placental abruption	A	2050	88.9	69.2	0.759	0.009
	B	2150	87.5	71.6	0.810	0.004
IUGR	A	2350	90	75.8	0.849	0.000
	B	1650	87.5	56.9	0.700	0.014
Transfer to NICU	A	1850	100	83.6	0.937	0.000
	B	1350	96.6	62.3	0.857	0.000

Perhaps Lindheimer's findings are near to truth that suggested that blood pressure, evidence of liver damage, blood system, and nervous signs are reliable determinants in the severity of preeclampsia [7]. The present authors suggest a whole aspect study to evaluate proteinuria correlation with each of pregnancy consequences.

Acknowledgement

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The effects of magnesium sulphate on the contractile activity of uterus in an animal model of preeclampsia

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Summary

Purpose: This study was undertaken to evaluate the effects of magnesium sulfate ($MgSO_4$) on the contractile activity of the uterus in a pregnant rat model of preeclampsia induced by N-nitro-arginine methyl ester (L-NAME). *Materials and Methods:* Twenty-eight, 160-220 gram, three to four month old female Sprague-Dawley rats were used in this study. After conception was confirmed by vaginal smears on the first day of pregnancy, the animals were allocated into four groups according to the chemicals fed in their drinking water as control (nothing administered), L-NAME (50 mg/kg L-NAME), $MgSO_4$ (600 mg/kg $MgSO_4$), and $MgSO_4$ + L-NAME group (600 mg/kg $MgSO_4$ + 50 mg/kg L-NAME). The pregnant uterus strips were isolated on the 19th day and the contractile activity of uterus was examined by applying 0, 0.1, 0.2, 0.4, 0.8, and 2.5 mIU/ml oxytocin to each group and responses are recorded accordingly. *Results:* There were no statistically significant differences regarding fetal parameters and peak amplitudes of the oxytocin stimulated pregnant rat myometrial strips among groups. In L-NAME group at 0 and 0.1 mIU/ml oxytocin, the contraction frequency in a ten-min period was statistically lower than the control group ($Z = -2.850$, $p = 0.004$; $Z = -2.902$, $p = 0.004$, respectively). In $MgSO_4$ group only at 0 mIU/ml oxytocin, the frequencies of the contractions in ten-min period were statistically lower than the control group ($Z = -2.973$, $p = 0.003$). In L-NAME + $MgSO_4$ group at 0, 0.1 and 0.2 mIU/ml oxytocin concentrations the frequencies of the contractions in ten-min period were statistically lower than the control group ($Z = -4.018$, $p = 0.000$; $Z = -3.237$, $p = 0.001$; $Z = -2.902$, $p = 0.004$, respectively). In L-NAME + $MgSO_4$ given group at each oxytocin concentrations, the frequencies of the contractions in ten-min period were lower but not statistically different than the L-NAME group. *Conclusion:* $MgSO_4$ has no significant effect on the amplitude of spontaneous or oxytocin induced myometrial contractions, but decreased the frequency of spontaneous contractions. At each doses of oxytocin, $MgSO_4$ has no significant effect on the frequency of contraction in a pregnant rat model of preeclampsia induced by L-NAME.

Key words: Magnesium sulfate; L-NAME; Uterus; Preeclampsia.

Introduction

Preeclampsia is a multi-system disorder characterized by hypertension and proteinuria in the last half of pregnancy and eclampsia refers to the development of grand mal seizures in a woman with preeclampsia, in the absence of other neurologic conditions that could account for the seizure. Although most affected pregnancies deliver at term or near term with good maternal and fetal outcomes, these pregnancies are at increased risk for maternal and/or fetal mortality or serious morbidity [1,2]. Preeclampsia occurs in up to 7.5 percent of pregnancies worldwide [3,4].

The pathophysiology of preeclampsia likely involves both maternal and fetal/placental factors. Abnormalities in the development of placental vasculature in early pregnancy may result in relative placental underperfusion/hypoxia/ischemia, which then leads to release of antiangiogenic factors into the maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations of the disease [5].

The optimal management of a woman with preeclampsia depends on gestational age and disease severity. The definitive treatment of preeclampsia is delivery, either by labor induction or cesarean section to prevent development of maternal or fetal complications from disease progression. Delivery results in resolution of the disease. In preeclampsia management, magnesium sulphate ($MgSO_4$) is the first-line treatment for the prevention of eclamptic seizures [6].

However, $MgSO_4$ is known to relax smooth muscle and is widely used as a tocolytic agent for preterm labor. If the tocolytic effect is significant at doses used for preeclampsia, $MgSO_4$ administration could increase the length of labor.

Several physiological mechanisms (neuronal, hormonal, metabolic, and mechanical) play a role in the control of myometrial activity during delivery. Alteration of uterine contractions by drugs or phytochemicals is of great importance in obstetrics practice, as it could lead to disruption of normal course of parturition [7].

Therefore the objective of this study is to investigate the effects of $MgSO_4$ on the contractile activity of uterus in a pregnant rat model of preeclampsia induced by N-nitro-arginine methyl ester (L-NAME).

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Materials and Methods

Pharmacologically induced model of preeclampsia

Nitric oxide (NO) is a potent vasodilator that is synthesized from the amino acid L-arginine, by nitric oxide synthase (NOS). Acute blockade of NO synthesis in studies using rats has demonstrated a marked rise in systemic blood pressure in these animals. Furthermore, chronic inhibition of NOS using N-nitro-arginine methyl ester (L-NAME) in pregnant rats led to the development of a model characterized by hypertension, proteinuria, reduced glomerular filtration rate, glomerular sclerotic injury, thrombocytopenia, and intrauterine growth restriction which is similar to preeclampsia in humans [8].

Animals and study design

The study was performed in accordance with the National Institutes of Health (NIH) Guidelines for the welfare and use of laboratory animals. The study protocol used was approved by the Kırıkkale University Ethics Committee (17.03.2006-01/06).

Adult, 160-220 gram, three to four months old, 28 female Sprague-Dawley rats were used in this study. Rats were maintained under controlled conditions. Food and water were freely available under 12 h light / 12 h dark cycle. The stage of the oestrus cycle was determined in each rat by vaginal cytological examination. Female rats were made pregnant by overnight pairing with males (two females : one male). To confirm pregnancy, the vaginal smears were checked twice daily early in the morning and evening for the presence of spermatozoa. The female rats were considered as pregnant after the determination of spermatozoa. After confirming the pregnancy (day 0 = spermatozoa positive), 28 pregnant rats were randomly allocated to four groups. First group was control group (n = 7) and allowed access to tap water. Second group, called L-NAME group (n = 7), received 50 mg/kg bw/day L-NAME hydrochloride, third group, called $MgSO_4$ group (n = 7), received 600 mg/kg bw /day $MgSO_4$, and fourth group, called L-NAME + $MgSO4_4$ (n = 7), received 50 mg/kg bw/day L-NAME + 600 mg/kg bw /day $MgSO_4$ in drinking water from 11^{th} to 19^{th} day of pregnancy. The systolic and diastolic blood pressure of pregnant rats was measured on day 12^{th} and 19^{th} by tail cuff device. Blood pressure was obtained from three consecutive measurements and average pressure value was recorded as the blood pressure of the rat at each time point. Test strips were used to detect the level of protein in urine. Systolic and diastolic blood pressures were increased in L-NAME given group when compared to control group on days 12 and 19. On day 19th proteinuria was seen in L-NAME given group. On day 19 of pregnancy, rats were anesthetized with sodium thiopental (Pentothal Sodium, Abbott, Turkey, 50 mg/kg). The abdomen was opened and two horns of the uterus were separated and freed from fat. Fetal tissues and placenta were separated. The number and the weight of fetuses were determined.

Isolated rat uterine strips

The uterine strips from each horn were mounted in Dale Solution (mM): NaCl: 154; KCl: 5,4; MgCl2: 0,024; glucose: 2,77; CaCl2: 1,63 and NaHCO3: 5,95. Uterine tissues were then sliced into four thin strips of approximately ten mm long from one pregnant rat. One end of the uterine strips, where the ovarium was located, were attached to force transducers and the other end was attached to a glass holder, under a resting tension of 500 mg in four-channel (ten ml) tissue baths. The tissue medium used was maintained at pH of 7.4, temperature of 37oC and gassed with carbogen (95% O_2 and 5% CO_2).

Table 1. — *Fetal parameters at day 19 of pregnancy.*

Parameters	Control (n = 7)	L-NAME (n = 7)	$MgSO_4$ (n = 7)	L-NAME +$MgSO_4$ (n = 7)
Weight of pregnant rats	222.57 ± 10.20	223.14 ± 6.29	221.71 ± 7.54	217.14 ±5.89
Number of fetuses	8.71 ± 0.81	10.14 ± 0.74	9.43 ± 0.48	8.71 ± 0.52
Total litter weight	19.78 ± 3.27	17.12 ± 1.59	19.62 ± 1.64	15.33 ± 1.08

Data were statistically analyzed using One Way ANOVA. Values are means ± SE; n = number of rats.

Experimental protocol

The uterine strips were washed at 15-minute intervals and left to equilibrate in bathing medium for one hour and the spontaneous contractions were observed. Following equilibration the viability of the strips were assessed by stimulating the uterus with ten mIU/ml oxytocin. The tissues were washed in five-minute intervals and observed for the recovery. Zero, 0.1, 0.2, 0.4, 0.8, and 2.5 mIU/ml oxytocin was applied to the tissue bath non-cumulatively. The contact time for each concentration was ten min. After each concentration the tissues were washed again at five-minute intervals and observed for recovery. Contractions were measured with a force displacement transducer and recorded.

Data analysis

The magnitude of uterine contractile responses to each concentration was expressed as mg tension, frequency as number of uterine contractions in ten minutes. The weights of the fetuses were expressed as grams.

Statistical analysis

Data processing was performed with the SPSS 15.0 package. The normality of all data was assessed by Shapiro-Wilk test. The frequency of the uterine contractions were distributed non-parametrically therefore tested using Kruskal Wallis test followed by the Mann- Whitney U test with Bonferroni adjustment to determine which of the four groups differed from each other. In case of fetal parameters of the pregnant rats and peak amplitude of the uterine myometrial contractions, one way ANOVA test was used. Differences were considered significant when $p < 0.05$ in One Way ANOVA and Kruskal Wallis test and $p < 0.0083$ in Mann Whitney U test with Bonferroni adjustment.

Results

Fetal parameters

Rats have multiple gestations so the total weight and total number of fetuses were considered to be the indicator of fetal growth. There were no statistically significant ($p > 0.05$) differences among the fetal parameters of trial groups (Table 1).

Myometrial strip contractions

In L-NAME group at 0 and 0.1 mIU / ml oxytocin concentrations, the frequencies of the contractions in ten-min period were statistically lower than the control group ($Z = -2.850$, $p = 0.004$; $Z = -2.902$, $p = 0.004$, respectively). In $MgSO_4$ group only at zero mIU/ml oxytocin concen-

Table 2. — *The effect of L-NAME, $MgSO_4$ and L-NAME + $MgSO_4$ on the frequencies of pregnant rat myometrial strips stimulated with oxytocin concentrations for 10 minutes.*

Oxytocin Concentrations	Control (n = 28)	L-NAME (n = 28)	$MgSO_4$ (n = 28)	L-NAME + $MgSO_4$ (n = 28)	*p*
0 mIU/ml	77.89[a]	51.80[b]	51.21[b]	45.09[b]	**
0.1 mIU/ml	74.41[ab]	48.89[cd]	56.52[bd]	46.18[d]	**
0.2 mIU/ml	72.38[a]	52.75[ab]	55.52[ab]	45.36[b]	*
0.4 mIU/ml	67.64	51.98	58.66	47.71	NS
0.8 mIU/ml	65.64	52.41	61.84	46.11	NS
2.5 mIU/ml	66.29	49.18	62.95	47.59	NS

Data were statistically analyzed using Kruskal Wallis test. Data that showed significant differences in Kruskal Wallis test * = $p < 0.05$, ** = $p < 0.01$ were then analyzed by Mann-Whitney U test to check differences between couples. Differences were considered significant when $p < 0.0083$ in Mann Whitney U test. The values were given as Mean rank. a, b, c, d: Mean Rank with in row with different superscript was significantly different according to Mann Whitney U test. NS = not significant. n = number of uterine strips.

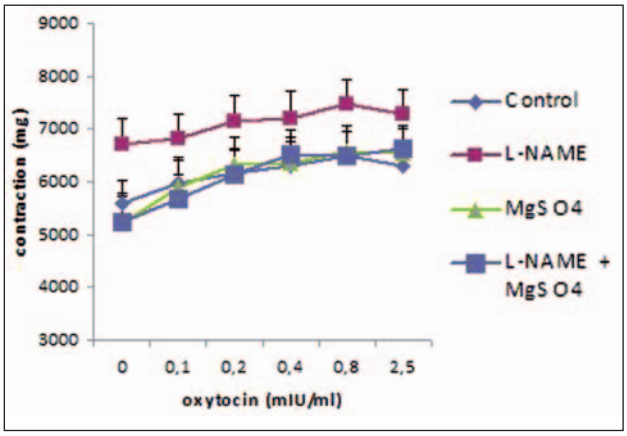

Figure 1. — The effect of L-NAME, $MgSO_4$ and L-NAME + $MgSO_4$ on the peak amplitudes of pregnant rat myometrial strips stimulated with oxytocin concentrations. Data were statistically analyzed by one way ANOVA and the values are given as mean ± standart error.

tration the frequencies of the contractions in ten-min period were statistically lower than the control group (Z = -2.973, $p = 0.003$). In L-NAME + $MgSO_4$ given group at 0, 0.1 and 0.2 mIU/ml oxytocin concentrations the frequencies of the contractions in ten-min period were statistically lower than the control group (Z = -4.018, p = 0.000; Z = -3.237, $p = 0.001$; Z = -2.902, $p = 0.004$, respectively). The effect of L-NAME, $MgSO_4$ and L-NAME+ $MgSO_4$ on the frequencies of pregnant rat myometrial strips stimulated with oxytocin concentrations for ten minutes were presented at Table 2. In L-NAME + $MgSO_4$ given group at each oxytocin concentrations, the frequencies of the contractions in ten-min period were lower but not statistically significant than the L-NAME group.

The peak amplitudes are presented in Figure 1. The peak amplitudes of the pregnant rat myometrial strips stimulated with oxytocin concentrations did not change among the trial groups.

Discussion

In the present study, there were no significant differences among the fetal parameters of trial groups. In contrast, in the study by Yallampalli and Garfield, 50 mg/kg bw/ day L-NAME decreased the weight of pups. They indicated that the mechanism for the low fetal weight in pregnant rats given L-NAME was not clear [9], but they believed that reduction of blood flow due to increased vasoconstriction of vessels by inhibition of the release of nitric oxide to the placental circulation caused this. In addition Pandhi et al. found a decrease in the weight of pups, and detected no difference on the number of the pups per rat [10].

This study showed that in inducted preeclampsia of pregnant rats the frequencies of uterus contractions were decreased compared to the control, but no difference was observed in the peak amplitude of the contractions. The inhibitory effect of preeclampsia on the frequency of uterine contraction was especially evident at low doses of oxytocin (0, 0.1 mIU/ ml). At higher doses this inhibitory effect somehow disappeared. In real clinical situations Szal *et al.* showed that in term nulliparous women, preeclampsia did not affect labor duration [11].

Magnesium sulfate is the first-line treatment for the prevention of primary and recurrent eclamptic seizures and prophylactic treatment with magnesium sulfate is indicated in all patients with severe preeclampsia [6]. Its mechanism of action is likely multi-factorial, encompassing both vascular and neurological mechanisms. Being a calcium antagonist, its effect on vascular smooth muscle is to promote relaxation and vasodilation and this may have a role in lowering total peripheral vascular resistance. In addition, $MgSO_4$ may have an effect directly on the cerebral endothelium by limiting vasogenic edema through decreasing stress fiber contraction and paracellular permeability via calcium-dependent second messenger systems such as myosin light chain kinase. Lastly, $MgSO_4$ may also act centrally to inhibit N-methyl-D-aspartate (NMDA) receptors, providing anticonvulsant activity by increasing the seizure threshold [12]

Magnesium sulfate is also widely used as the primary tocolytic agent. The ability of $MgSO_4$ to inhibit uterine contractility both in vivo and in vitro has been appreciated [13]. It also has neuroprotective properties and free radical reducing effects [14].

The mechanisms and time required for pharmacologic concentrations of $MgSO_4$ to inhibit myometrial contractility remains in question. Magnesium inhibits extracellular calcium entry, intracellular calcium release, cytosolic calcium oscillations, and phasic contractions of myometrial smooth muscle [15, 16].

In the present study, $MgSO_4$ did not change the spontaneous and oxytocin-induced myometrial amplitude of contraction, but without oxytocin, the presence of $MgSO_4$ alone depressed the frequency of contractions. Therefore in vitro, frequencies of uterine contractions are decreased but amplitudes are maintained, so possible tocolytic effect of $MgSO_4$ is by inhibition of spontaneous myometrial contractility through decreased frequency of contraction. In accordance, Kantas *et al.* showed $MgSO_4$ reduced the frequency of spontaneous contractions without affecting the amplitude in isolated myometrial strips of pregnant human and rat [17].

Uterine contractile activity is determined by the increase in intracellular free Ca^{2+} concentration in the myometrial cells [18], and oxytocin stimulates uterine contractions by two receptor mediated mechanisms, a second messenger system involving phospholipase C, which results in release of calcium from intracellular stores and the opening of calcium channels with the resultant calcium influx [19]. In an in-vitro study made by Tica *et al.* $MgSO_4$ temporarily reduced spontaneous myometrial contractions in a dose-dependent manner, with efficient regimens at 2.0-2.5 mM oxytocin-induced contractions were reduced by 30% - 40% at eight mM and decreased further at 9-10 mM [20]. Induced contractions were reduced, in a dose-dependent and time-dependent manner (maximum effect at 20 min), at higher Mg^{2+} concentrations and with non-significant proportional differences between pregnant and non-pregnant myometrium. As a conclusion, the authors decided that $MgSO_4$ acts in the inhibition of spontaneous myometrial contractility, but not of uterine-induced hyperactivity [20].

In the present study the authors showed that, in L-NAME induced preeclampsia of rats in vitro changed the frequency of the spontaneous uterine smooth muscle contractions but did not affect the maximum oxytocin contractility responses. The inhibitory effect on the frequency of uterine contraction was especially seen at low doses of oxytocin (0, 0.1 mIU/ml) in a pregnant rat model of preeclampsia induced by L-NAME. At higher doses this inhibitory effect somehow disappeared. This inhibitory effect was seen in $MgSO_4$ given group only on spontaneous contractions and in L-NAME+ $MgSO_4$ group at doses 0, 0.1 and 0.2 mIU/ml.

The most important finding in this study is that, at each doses of oxytocin, $MgSO_4$ has no significant effect on the frequency of contraction in a pregnant rat model of preeclampsia induced by L-NAME. In clinical situations also, there is no evidence that $MgSO_4$ therapy prolongs the duration of normal labor [11, 21, 22]. Witlin *et al.* showed that the use of magnesium sulfate during labor in women with mild preeclampsia at term does not affect any component of labor but did necessitate a higher dose of oxytocin [23].

In conclusion, $MgSO_4$ has no significant effect on the amplitude of spontaneous or oxytocin induced myometrial contractions, but decreased the frequency of spontaneous contractions. At each dose of oxytocin, $MgSO_4$ has no significant effect on the frequency of contraction in a pregnant rat model of preeclampsia induced by L-NAME.

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Alteration of T-cell subpopulations and lipid peroxidation in the blood of patients with vulvar non-neoplastic epithelial disorder

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Summary

Objective: To determine the relationship between vulvar non-neoplastic epithelial disorder and thymus-dependent lymphyocyte levels and lipid peroxidation. *Materials and Methods*: the authors measured the levels of CD3+, CD4+, CD8+, CD16+ T cell, and the concentration of superoxide dismutase (SOD) and malondialdehyde (MDA) in the blood of 62 patients with vulvar non-neoplastic epithelial disorder. A control group consisted of 30 normal women from the present hospitals. *Results:* The level of CD4+/CD8+ T-lymphocytes and SOD in the blood of the patients with vulvar non-neoplastic epithelial disorder was significantly lower than that in control subjects, but the level of MDA was higher as compared with normal women. *Conclusion:* There is increased immune activation and lipid peroxidation in patients with vulvar non-neoplastic epithelial disorder, which could contribute to destruction of vulvar tissue.

Key words: Vulvar non-neoplastic epithelial disorder; Thymus-dependent lymphyocyte; Lipid peroxidation; Superoxide dismutase.

Introduction

Non-neoplastic epithelial disorder of vulva, also known as vulvar white lesions, including squamous cell hyperplasia, lichen sclerosus (LS), lichen planus and lichen simplex chronicus, etc., is a form of dermatosis characterized by ivory-colored, severe pruritus and decreased tissue elasticity of the vulva and perianal skin. The cause remains uncertain, and the disorder is difficult to cure [1,2]. To investigate immune status and potential mechanisms of tissue injury in patients with vulvar non-neoplastic epithelial disorder, the authors determined levels of CD3+, CD4+, CD8+, and CD16+ T cell subsets, and assessed parameters relevant to peroxidation, including expression levels of superoxide dismutase (SOD) and malondialdehyde (MDA). The purpose of this study was to expand the authors' understanding of the potential role of the immune system and lipid peroxidation in patients with vulvar non-neoplastic epithelial disorder, to find the evidence of immune and lipid peroxidation-mediated impairment of cells, and to explore the pathogenic mechanism of vulvar non-neoplastic epithelial disorder.

Materials and Methods

The authors recruited 62 patients with vulvar non-neoplastic epithelial disorder who had been treated in the vulvar disease policlinic of the present hospitals from June 2001 to December 2002. Mean age of patients was 37.8 ± 9.2 years. All cases were identified histologically, and included 26 cases of squamous hyperplasia (SH), 23 cases of lichen sclerosus (LS), and 13 cases mixed histological type. In all cases, immune disease and acute and chronic diseases affecting patients' lipid peroxidation were excluded by clinical history and physical examination. Patients had no drug history affecting their immune status or lipid peroxidation condition. Mean age of the control group of 30 normal women was 39.2 ±10.6 years, which was not statistically different from patients ($p > 0.05$).

In all patients, six ml of venous blood was obtained by phlebotomy in the morning after an overnight fast. Of the six ml total, three ml was treated with an anticoagulant for detection of T-cell subsets (CD3+, CD4+, CD8+ and CD16+). The remaining three ml of blood was centrifuged, and serum was separated and stored in a refrigerator at 0-4^{0}C for measurement of SOD and MDA.

CD3+, CD4+, CD8+, and CD16+ T cells were examined with an immunofluorescence flow cytometer; using the fluorochrome propidium iodide and an RNA enzyme.

SOD activity was determined by the nitrite method. Nitrite unit per milliliter of serum acts as active unit of SOD. Reagent and colored immune board were provided by the Molecular Biology Center of China PLA Navy General Hospital.

MDA activity was examined using thiobarbituric acid (TBA) methods. The red product derived from compound of MDA and TBA has the highest absorption peak at 532 nm, the content (μmol/L) and can be measured with a 722 spectrometer.

Statistical analysis included Student's t-test, analysis of variance and linear correlation. A p value less than 0.05 was considered significant.

Results

There appeared to slightly lower levels of CD3+ and CD4+ T cells and slightly higher levels of CD8+ and CD16+ T cells in patients compared to controls, but these differences were not statistically significant. However, the CD4+/CD8+ ratio was significantly lower in patients compared to controls ($p < 0.05$, Table 1).

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Table 1. — *T lymphocyte subsets in patients with vulvar non-neoplastic epithelial disorder compared to control subjects.*

	CD3+	CD4+	CD8+	$CD4^+/CD8^+$	CD16
Control	67.63±9.65	37.01±7.69	25.35±6.75	1.55±0.58	16.53±7.64
Vulvar white lesions	63.58±10.49	34.5±6.58	28.57±9.37	1.23±0.51	18.24±6.7
p	>0.05	>0.05	>0.05	<0.01	>0.05

Serum levels of SOD in patients were significantly less than those of the control group, however serum MDA levels were higher in patients compared to controls ($p < 0.05$ for both comparisons, Table 2).

There were no significant differences detected among different types of vulvar non-neoplastic epithelial disorder in blood level of T cell subsets, serum SOD, or serum MDA ($p > 0.05$ for all comparisons).

Linear correlation analysis demonstrated a significant positive correlation between serum levels of SOD and CD4+/CD8+ ratio, CD3+ or CD4+, as well as MDA and CD8+ or CD16+ ($r = 0.66$, $p < 0.05$ for all comparisons).

Table 2. — *Serum levels of SOD and MDA in patients with vulvar non-neoplastic epithelial disorder compared to control subjects.*

	n	SOD	MDA
Control	30	23.81±4.53	3.25±0.58
Vulvar white lesions	62	18.24±3.36	4.01±0.64
p		<0.01	<0.01

Discussion

Vulvar non-neoplastic epithelial disorder is a degeneration and pigmental change caused by dystrophy of the skin and mucous membranes of the vulva. The exact cause is unclear, but is probably related to localized nerve and vascular dysfunction, stimulation of epidermal metabolites, and lack of estrogen, but the potential relationship with infection, immunity, and abnormal expression and/or function of SOD has not been well-studied and available data are conflicting [1-4].

Cluster of differentiation (CD) antigens are cell surface proteins or glycoproteins appearing or disappearing in lineage-specific leukocytes during specific stages of differentiation and activation. Besides serving to identify cell lineage and stage of development and activation, CDs extensively participate in development, maturation, differentiation, growth, migration and activation of cells, promote the interaction of immune molecules, and regulate cell-matrix adhesive interactions [5,6]. The principle function of CD3 in leukocytes is to stabilize the structure of TCRs and transmit signals activating T cells. When T cell receptors (TCRs) recognize and bind antigens, cells expressing CD3 participate in delivering signals to cytoplasm of T cells as the first signal inducing T cell activation. CD4 antigen is expressed on the cell surface of some T lymphocytes, thymus cells, B lymphocytes, B cells transformed by Epstein-Barr (EB) virus, monocyte / macrophages and brain cells, regulates adhesion and signal transduction, and participates in the pathogenesis of most autoimmune diseases. CD4 antigen mainly plays a role in the elimination of activated activated protein Cs (APCs) and T cells, thereby limiting the immune response. CD8+ cells can induce adhesion of cells and act as signal transduction molecules, but mainly recognize and kill tumor cells and virus-infected cells. With the development of modern immunology, much has been regarding about T cells surface markers and their biological function. Both CD4+ and CD8+ cells include subsets which can induce either up- or down-regulation. By interactions among various cell types (especially various T cells subsets), the organism can maintain normal immune status and induce an appropriate immune response to eliminate foreign pathogens, while not causing harm to the host organism. Usually, the total numbers of T cells and T cell subsets is relatively constant. If the total T cells or the ratio of CD4+/CD8+ changes, the function of immune regulation can be considered abnormal. CD16 antigen is a surface marker expressed by natural killer (NK) cells. NK cells are special lymphocytes, are different from T and B lymphocytes, and play an important role in killing target cells. The lethal effect on target cells of NK cells is rapid; NK cells can destroy target cells in less than four hours, and this process does not require advance sensitization. The target cells include tumor cells, viruses, bacterium-infected cells, and some normal cells [7].

Our study found a trend towards a decrease in levels of CD3+ and CD4+ T cells in patients with vulvar non-neoplastic epithelial disorder, though the difference compared to control subjects was not statistically significant. This may be related to an insufficiency in immune ability to remove activated APCs and T cells, leading to local pathological changes. The present results indicate there may be an excessive immune response that leads to pathologic damage and is reflected by increasing levels of CD8+ and CD16+ cells, and decreasing CD4+/CD8+ cell ratios in patients with vulvar non-neoplastic epithelial disorder.

In the course of normal metabolism, oxygen-free radicals are produced as the byproducts of enzymatic and non-enzymatic reactions, and can attack unsaturated fatty acids in cell membranes and cause lipid peroxidation. Lipid peroxides may interfere with normal cell metabolism and function. Antioxidants that protect against oxygen-free radical damage include SOD and various superoxidases. SOD has an extremely important effect on the balance of oxidation and reduction, and can rapidly dismute 2 superoxide anion radicals, thus reducing oxidant stress and minimizing the

potential for cellular damage. When local expression of SOD in skin tissues is decreased, free radicals can more readily form and accumulate. Increased local concentrations of free radicals will tend to damage cells and tissues of collagenous fibers, the reticular fibers, the elastic fibers, and a number of macromolecules within blood vessels and nerves including proteins, nucleic acids, and lipids, and this in turn could promote dystrophy of vulva by destroying the physiological structure, metabolism, and sources of nutrition for vulvar tissues [8-11]. In this study, levels of MDA were significantly increased and SOD was obviously decreased in patients with vulvar non-neoplastic epithelial disorder. These results suggest that lipid peroxidation was promoted, and antioxidation defenses diminished in patients with vulvar non-neoplastic epithelial disorder. The increase in oxygen-free radicals and their metabolites that would be expected to result from this imbalance would tend to damage the skin and mucus of the vulva of patients.

In conclusion, the results of the present study suggest that patients with vulvar non-neoplastic epithelial disorder simultaneously exhibit excessive immunoreaction and increased production of free radicals, raising the possibility that these two effects combine to importantly contribute to pathologic features of vulvar non-neoplastic epithelial disorder. The authors found no significant differences in these parameters between different tissue types of vulvar non-neoplastic epithelial disorder, suggesting that they may similar immune mechanisms contribute to different forms of vulvar non-neoplastic epithelial disorder. Differences in pathological behavior are likely to related to other factors in the progression of the disease.

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The association between inherited thrombophilia and recurrent pregnancy loss in Turkish women

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Summary

Objective: To investigate the relation between recurrent pregnancy loss (RPL) and factor V Leiden, prothrombin G20210A, and C677T methylenetetrahydrofolate reductase (MTHFR) mutations. *Materials and Methods:* A case-control study was conducted on 95 consecutive cases with RPL, and 40 age-matched controls who had no history of pregnancy loss and had at least one successful pregnancy. After application of exclusion criteria, 60 patients in the study group and 40 control cases were compared for thrombophilic factors. *Results:* Thirteen out of 60 RPL cases and one out of 40 in the control group were carriers of factor V Leiden mutation. While six patients were carriers of prothrombin G20210A gene mutation, none in the control group carried this mutation. Twenty-nine out of 60 RPL cases and 17 out of 40 control cases had MTHFR mutation. *Conclusion:* The authors found a positive correlation between RPL and FVL and FII gene mutations, but no significant association between RPL and MTHFR gene mutation.

Key words: Recurrent pregnancy loss; Factor V Leiden; Prothrombin G20210A mutation; MTHFR C677T mutation.

Introduction

Recurrent pregnancy loss (RPL) is a frequent health problem, with three or more losses affecting one to two percent and two or more losses affecting up to five percent of women in the reproductive age [1, 2]. While several etiologies have been implicated to play a role in RPL including chromosal translocations and inversions, anatomic alterations of the uterus, endocrinologic abnormalities, and autoimmune disorders [3, 4], until recently the majority of RPL remained unexplained. Association with acquired thrombophilia, such as antiphospholipid antibodies and RPL, is well established. Based on the histological findings of extensive infarction and necrosis in the placentas of women with antiphospholipid syndrome, researchers postulate that uteroplacental thrombosis may lead to placental infarction and eventual fetal death [5]. A number of studies in women with inherited thrombophilia have also suggested an association with fetal loss.

The three most common genetic thrombophilias known to predispose to venous thrombosis are: factor V Leiden (FVL), methylenetetrahydrofolate reductase mutation (MTHFR, C677T) [6, 7], and prothrombin gene mutation (FII, G20210) [8]. FVL mutation involves a G→A substitution at nucleotide 1691 of coagulation factor V gene [9]. Factor Va becomes resistant to degradation by activated protein C due to this substitution. This mutation in the factor V gene increases the risk of venous thromboembolism three- to five-fold in heterozygous individuals [10]. One genetic variation, a G to A transition at nucleotide position 20210, in the 3'-untranslated region of the coagulation factor II gene, has been found to be associated with increased prothrombin levels and risk for venous thrombosis [8]. This mutation is quite common in the normal population (0.7% - 4.0%) [11], whereas it is responsible for 6.2% [8] of all the cases of thromboses. MTHFR deficiency is the most common congenital error of folate metabolism, which leads to elevated homocysteine plasma levels. A common mutation in the MTHFR gene, i.e. a cytosine to thymine transition at position 677, is associated hyperhomocysteinemia which predisposes to thrombosis [12, 13].

The aim of this study was to evaluate the prevalence of FVL, prothrombin G20210A, and C677T MTHFR mutations in women with recurrent fetal loss in the Turkish population.

Materials and Methods

This study was performed in Ataturk University Faculty of Medicine, Department of Obstetrics and Gynecology, between January 2007 and March 2008. In this case-control study the prevalence of factor V Leiden, prothrombin G20210A and C677T MTHFR mutations were determined in a consecutive series of 95 women referred for evaluation of recurrent spontaneous pregnancy loss (study group patients) and 40 women with at least one successful pregnancy and no history of pregnancy loss (controls).

The patients with recurrent pregnancy loss were Turkish women (age range 19-46 years; mean 29.14 ± 6.18), referred for evaluation at a university hospital. The clinical details of each patient and her pregnancy losses were recorded, paying particular attention to whether the previous pregnancy losses occurred in the early pregnancy period (first trimester, ≤ 12 weeks of gestation) or late pregnancy period (> 12 weeks of gestation), and whether

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Table 1. — *Charactarictics of RPL patients with additional pathology.*

	RPL group with additional pathology (n = 35)	FII G20210A carriers (n = 2)	FVL mutation carriers (n = 2)	MTHFR mutation carriers (n = 15)
Age (years; mean)	30.1 ± 6.3	33.5 ± 7.8	27.5 ± 3.5	30.9 ± 6.3
Defined causes				
Anatomical	7	1	2	0
Hormonal	5	0	0	1
Chromosomal	2	1	0	1
Autoimmune	13	0	0	2
Other coagulation disorders*	3	0	0	3
Different combination of these causes	5	0	0	8
Total	35	2	2	15

*Deficiencies of antithrombin III, protein C and protein S.

Table 2. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between the RPL patients and the controls.*

Type of genetic defect	Recurrent pregnancy loss (n = 60)	Controls (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	13 (21.7)	1 (2.5)	10.8 (1.35-86.16)	0.007
Prothrombin G20210A mutation n (%)	6 (10)	0 (0)	Not calculated	0.039
Either mutation n (%)	19 (31.7)	1 (2.5)	18.07 (2.31-141.53)	<0.001

the patients were primary or secondary RPL (primary RPL are women with no previous live births, secondary if there was a live birth followed by pregnancy losses. Eligibility criteria for the study group was a history of two or more spontaneous pregnancy losses. Forty-six women had two pregnancy losses, 34 had three, and 15 had more than three. Sixty-eight out of 95 patients had only early, five had early and late, and 22 had late pregnancy losses. All women had been previously investigated for autoantibodies, glucose tolerance test, HbA1C levels, thyroid function, serum prolactin levels, coagulation disorders other than factor V Leiden, MTHFR and prothrombin G20210A polimorphism, uterine anatomic anomalies with hysterosalpingography (HSG,) and karyotype of both parents. Of these women; two had abnormal karyotype, seven had uterine septum, four were positive for antiphospholipid antibodies (APA), nine had autoantibodies other than APA, 13 had different combination of other pathologies, such as diabetes mellitus, thyroid dysfunction, hyperprolactinemia, and deficiencies of antithrombin III, protein C, and protein S (Table 1). None of the patients had a history of thrombo-embolic event.

The control group consisted of 40 age-matched women (age range 19-45 years, mean 30.50 ± 6.77) with no previous pregnancy loss and thrombo-embolic events. Both study patients and control subjects were born in the east of Turkey and were living in Erzurum province or a nearby region.

Total genomic DNA was isolated from peripheral vein blood samples with a MagNA Pure LC DNA Isolation Kit using a MagNA Pure LC 2.0 Automated DNA isolation instrument . The FVL, Factor II, and MTHFR kit allowed mutation genotyping using a Lightcycler 2.0 Instrument.

Data were stored and analysed using SPSS (Statistical Package for Social Science, release 15.0) in an IBM-compatible computer. The chi-square and Student's t test were used to assess intergroup significance. In addition, the odds ratios (OR) and 95% CI were estimated separately for each polymorphism. The difference was considered as statistically significant when $p < 0.05$.

Results

Thirty-five patients out of 95 were not included in the statistical analysis because they had additional pathology and remaining 60 were included in order to investigate the relationship between the RPL and FVL, prothrombin G20210A and C677T MTHFR mutations. Mean age of the RPL group without additional pathology and the control group was 28.57 ± 6.1 and 30.50 ± 6.8 years, respectively ($p > 0.05$). The 60 patients in the study group had 177 previous pregnancy losses (mean: 2.95 ± 1.65). Forty patients out of 60 (66.7%) were diagnosed as having at least one thrombophilia marker, whereas 20 (33.3%) had no thrombophilia.

Concerning the FVL mutation, 13 out of 60 RPL patients and one out of 40 controls carried FVL mutation (21.7 vs. 2.5%, $p = 0.007$, odds ratio 10.8, 95% CI: 1.35 - 86.16). No factor V Leiden homozygosity was found in the RPL and control groups (Table 2). Forty-six out of 60 RPL patients had early and 14 had late pregnancy losses. 11 out of 46 patients with early pregnancy loss, and one out of 40 controls carried FVL mutation (23.9 vs 2.5%, $p = 0.004$, odds ratio 12.26, 95% CI: 1.51 - 99.83). Two out of 14 patients with late pregnancy loss and one out of 40 controls carried FVL mutation (14.3 vs 2.5%, $p = 0.09$, odds ratio 6.5, 95% CI: 0.5 - 78.1). The prevalence of FVL mutation was higher in the group of late pregnancy loss, but the difference did not reach statistical significance (Table 3). Of the entire study group of 60 women, 41 were primary RPL, whereas 19 were secondary RPL. Twelve out of 41 patients who had primary RPL and one out of 40 controls carried the FVL mutation (29.3 vs 2.5%, $p = 0.001$, odds ratio 16.14, 95% CI: 1.98 - 131.24). One out of 19 patients who had secondary RPL and one out of 40 controls carried the FVL mutation (5.3 vs 2.5%, $p = 0.3$, odds ratio 2.17, 95% CI: 0.13 - 36.62). The prevalence of FVL mutation was higher in the group of secondary RPL, but the difference did not reach statistical significance (Table 4). Thirteen out of 60 RPL patients without additional pathology and two out of 35 patients with additional pathology carried the FVL mutation (21.7 vs 5.7 %, $p = 0.040$, odds ratio 4.56, 95% CI: 0.97 - 21.59) (Table 5).

Concerning the prothrombin G20210A polymorphism, six prothrombin G20210A mutations were observed in the RPL group, whereas none of the controls had prothrombin G20210A mutation (10% vs 0%, $p = 0.039$, odds ratio was not calculated since none of the controls had prothrombin G20210A mutation) (Table 2). No prothrombin (FII)

Table 3. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with early and late RPL patients and the controls.*

Type of genetic defect	Early RPL (n = 46)	Controls (n = 40)	Odds ratio (95% CI)	*p* value	Late RPL (n = 14)	Controls (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	11 (23.9)	1 (2.5)	12.26 (1.51-99.83)	0.004	2 (14.3)	1 (2.5)	6.5 (0.5-78.1)	0.09
Prothrombin G20210A mutation n (%)	5 (10.87)	0 (0)	Not calculated	0.032	1 (7.14)	0 (0)	Not calculated	0.089
Either mutation n (%)	16 (34.8)	1 (2.5)	20.8 (2.6-165.8)	< 0.001	3 (21.4)	1 (2.5)	10.6 (1.0-112.7)	0.02

Table 4. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with primary and secondary RPL patients and controls.*

Type of genetic defect	Primary RPL (n = 41)	Controls (n = 40)	Odds ratio (95% CI)	*p* value	Secondary RPL (n = 19)	Controls (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	12 (29.3)	1 (2.5)	16.14 (1.98-131.2)	0.001	1 (5.3)	1 (2.5)	2.17 (0.13-36.62)	0.3
Prothrombin G20210A mutation n (%)	5 (12.2)	0 (0)	Not calculated	0.023	1 (5.26)	0 (0)	Not calculated	0.14
Either mutation n (%)	17 (41.5)	1 (2.5)	27.6 (3.45-221.1)	< 0.001	2 (10.5)	1 (2.5)	4.59 (0.39-54.09)	0.19

Table 5. — *Comparison of the prevalence of factor V Leiden and prothrombin G20210A mutations between women with and without additional pathology.*

Type of genetic defect	RPL patients without additional pathology (n = 60)	RPL patients with additional pathology (n = 40)	Odds ratio (95% CI)	*p* value
Factor V Leiden mutation n (%)	13 (21.7)	2 (5.7)	4.56 (0.97-21.59)	0.040
Prothrombin G20210A mutation n (%)	6 (10)	2 (5.7)	1.83 (0.35-9.62)	0.47
Either mutation n (%)	19 (31.7)	4 (11.4)	3.59 0.026 (1.11-11.63)	

Table 6. — *Comparison of the prevalence of C677T methylenetetrahydrofolate reductase mutation between RPL patients and controls.*

Type of genetic defect	RPL patients (n = 60)	RPL patients (n = 40)	Odds ratio (95% CI)	*p* value
C677T methylenetetrahydrofolate reductase mutation n (%)	29 (48.3)	17 (42.5)	1.27 (0.56-2.83)	0.57
Homozygous n (%)	1 (1.67)	3 (7.5)	0.21 (0.02-2.09)	0.15
Heterozygous n (%)	28 (46.67)	14 (35)	1.63 (0.71-3.71)	0.25

G20210A homozygosity was found in the RPL group. Five out of 46 patients with early pregnancy loss (10.87% vs 0%, $p = 0.032$) and one out of 14 patients with late pregnancy loss (7.14% vs 0%, $p > 0.05$) carried the FII G20210A mutation, whereas none of the controls had prothrombin G20210A mutation. The prevalence of FII G20210A mutation was higher in the group of late pregnancy loss, but the differences did not reach statistical significance (Table 3). Five out of 41 patients who had primary RPL (12.2% vs 0%, $p = 0.023$) and one out of 19 patients who had secondary RPL (5.26% vs 0%, $p > 0.05$) carried the FII G20210A mutation, whereas none of the controls had prothrombin G20210A mutation (Table 4). Six out of 60 RPL patients without additional pathology and two out of 35 patients with additional pathology carried the FII G20210A mutation (10% vs 5.7%, $p > 0.05$) (Table 5).

Concerning the C677T MTHFR mutation, 29 out of 60 RPL patients and 17 out of 40 controls had C677T MTHFR mutation (48.3% vs 42.5%, $p = 0.566$, odds ratio:1.27, 95% CI: 0.56 - 2.83). Among the RPL patients with C677T MTHFR mutation, only one patient was homozygote and the rest of the patients (n = 28) were heterozygote, whereas three patients out of 17 controls with C677T MTHFR mutation were homozygote and the remaining 14 were heterozygote (Table 6).

Two women with RPL were compound heterozygote, i.e. carrier of both the FII G20210A and C677T MTHFR mutation, whereas six RPL women were compound heterozygote, i.e. carrier of both the FVL and C677T MTHFR mutation.

These results suggest that factor V Leiden and prothrombin G20210A mutation, but not C677T MTHFR mutation, may be predisposing factors for RPL and that the prevalence of both FVL and prothrombin G20210A mutation are more prominent in early and primary RPL patients.

In order to investigate whether women with three or more RPL more frequently carry the FVL and prothrombin G20210A mutations than women with only two RPL, the prevalence of these two mutations is compared between the RPL patients and the controls. Five out of 28 women with two RPL (17.9%) and eight out of 32 with three or more

(25%) carried the FVL mutation ($p = 0.50$). Three out of 28 women with two RPL (10.7%) and three out of 32 with three or more (9.4%) carried the prothrombin G20210A mutation ($p = 0.86$).

Discussion

This study revealed a strong positive relationship between factor V Leiden mutation and fetal loss (odds ratio: 10.8). FVL mutation is a common genetic defect and its prevalence was reported as four percent in Caucasians and 4.3% in the Greek population [14, 15].

A meta-analysis reported an odds ratio of 2.0 in terms of association between factor V Leiden and factor II mutations and RPL [16]. In their study on Jewish women, Brenner *et al.* reported that frequency of FVL and factor II mutations were significantly higher in their study group compared to controls (32% - 10% and 8% - 4%, respectively) [17]. In a study performed in Greek population, Foka *et al.* reported significantly higher frequencies of FVL and FII mutations in their study group compared to controls (19% - 4%, $p = 0.003$, OR = 5.5 vs 9%- 2%, $p = 0.038$, OR = 4.6) [18]. In terms of association between FVL and FII mutations and RPL, a study performed by Settin *et al.* revealed odds ratios of 21.38 vs 36.7, respectively [19]. In the present study, the authors found an odds ratio of 10.8 in terms of association between FVL and RPL (21.7%-2.5%, $p = 0.007$, odds ratio = 10.8). Although FII gene mutation prevalence was high in the study group, since there was no case with FII gene mutation in the study group, odds ratio calculation was unavailable (10% - 0%, $p = 0.039$).

Zammiti *et al.* and Mtiraoi *et al.* reported relatively higher FVL mutation rates in their study groups compared to controls, however FII mutation was not significantly higher in Tunisian patients [20, 21]. Also, Grandone *et al.* reported higher FVL mutation rates in affected Italian women (16.28% - 4.24%, $p = 0.011$) [22]. On the contrary to the above studies, in their study on Turkish women with RPL, Sehirali *et al.* observed more significantly higher frequencies of FII mutations than FVL mutations in their study group compared to controls [23]. Meanwhile, some studies found no association between RPL and FVL and FII mutations [12, 24-29].

In a meta-analysis, Rey *et al.* reported a positive relationship between FVL mutation and both early and late pregnancy losses, while they found FII mutation was relatively more associated with early pregnancy losses [30]. Reznikoff-Etiévant *et al.*, in their study which included 260 Caucasian women with two or more concomitant pregnancy losses before ten weeks gestation, reported that FVL mutation was significantly associated with RPL before ten weeks gestation [31]. Krause *et al.* also reported significantly high frequencies of FVL mutations in German women with early pregnancy losses [32]. Meanwhile, some studies reported that FVL mutation was found in higher frequencies among cases with late pregnancy losses [22, 33, 34]. There are many sudies which present evidence of a strong association between FII gene mutation and late pregnancy losses [17, 32-35]. In the present study, the authors found that FVL mutation was more frequent in cases with early pregnancy loss (23.9%-2.5%, $p = 0.004$, odds ratio: 12.26). Although prevalance of FVL mutation was higher in cases with late pregnancy loss, it did not reach statistical significance (14.3%-2.5%, $p = 0.09$, odds ratio: 6.5). FII mutation was also found as more frequent in cases with early RPL, however odds ratio could not be calculated since there were no cases with FII gene mutation in women in the control group (10.87%-0%, $p = 0.032$). FII gene mutation prevalance was also higher in cases with late RPL, however it was statistically insignificant (7.14% - 0%, $p > 0.05$).

Kutteh *et al.* found no statistical difference between groups with primary and secondary RPL in terms of FVL mutation prevalence [36]. In the present study, while FVL mutation prevalence was significantly higher in the group with primary RPL (29.3% - 2.5%, $p = 0.001$, odds ratio = 16.14), it was higher but statistically insignificant in the group with secondary RPL compared to the control group (5.3%-2.5%, $p = 0.3$).

MTHFR deficiency is a metabolic disease, which is thought to cause placental infarcts associated with arterial and venous thromboemboli, has been studied in women with RPL. Brenner *et al.* and Kutteh *et al.* reported no association between MTHFR C677T gene mutation homozygosity and RPL [17, 36]. In their meta-analysis, Rey *et al.* found no significant association between MTHFR mutation homozygosity and RPL [30]. Habibovic *et al.* reported no significant association between MTHFR C677T gene mutation and RPL, as a result of their study on Turkish population [37]. The present authors also did not find any significant association between MTHFR gene mutation and RPL (48.3%-42.5%, $p = 0.566$, odds ratio = 1.27).

Conclusion

Results of the present study showed that RPL was associated with FVL and FII gene mutations but not with MTHFR mutation. The results also propose that FVL and FII gene mutations may be predisposing factors for RPL, especially for early and primary RPL.

In addition, the present study revealed no significant difference between cases with three or more RPL and cases with only two concomitant pregnancy losses, in terms of FVL and FII gene mutation prevalence.

Paucity of cases included to the control group was a limitation of this study. In the literature, there is no consensus on the association between RPL and thrombophilic factors. Thus, properly-designed further studies which include larger numbers of women are needed to illuminate this subject.

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Hypothyroidism and first-trimester spontaneous miscarriages

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Summary

Objective: To evaluate the association between hypothyroidism and first-trimester spontaneous miscarriages and to explain the mechanism. *Materials and Methods:* Patients admitted between October and May 2011 with threatened miscarriage in the first trimester were analyzed and levels of progesterone and thyroid hormones as T3, T4, and thyroid-stimulating hormone (TSH) were estimated. Once hypothyroidism was diagnosed, patients were treated with sodium levothyroxine (LT4) as substitution and outcomes were observed. *Results:* Measurement of progesterone was useful for predicting the outcome of threatened miscarriage The results showed that progesterone (P) = 14.74 ng/ml is selected as predictive value to judge whether the fetal treatment was successfully or not. When serum P value is above 14.74ng/ml before treatment, it may favour a miscarriage, if the serum P value is below 14.74 ng/ml, miscarriage is unlikely; its sensitivity and specificity are high. The risk for miscarriage in patients diagnosed with hypothyroidism in which LT4 substitution was similar to the level observed in the controls, and P between the two groups had no distinct difference. The mechanism explaining the risk of miscarriage increased by thyroid disorders remains unclear, which needs advanced research. *Conclusion:* Screening of thyroid disorders has important clinical significance in early pregnancy, and substitution of LT4 to those who are in the early pregnancy with hypothyroidism could reduce the risk of miscarriage.

Keywords: Hypothyroidism; Pregnancy; Miscarriage; Thyroid autoimmunity; Progesterone.

Introduction

The incidence of hypothyroidism in women of child bearing age is approximately 2% to 4% [1,2], which has an increasing tendency in recent years. The endocrine function of the thyroid and autoimmune disorders can significantly affect the pregnancy itself, leading to adverse pregnancy outcomes [3,4]. Abortion is one of the early complications, but the mechanism explaining the risk of miscarriage increased by thyroid disorders remains unclear. Most scholars agreed that once hypothyroidism was diagnosed, patients should be treated with sodium levothyroxine (LT4) as substitution as soon as possible, as early diagnosis and treatment can ameliorate pregnancy outcomes. In this study, thyroid disorders were screened in patients with threatened miscarriage in the first trimester. Once hypothyroidism was diagnosed, the patients were treated with LT4 as substitution, and outcomes were observed. References that pathological mechanisms explain the risk of miscarriage increased by hypothyroidism were reviewed at home and abroad.

Materials and Methods

General information

As shown in Table 1, between October, 2010, and March, 2012, a total of 164 women underwent threatened miscarriage in the first trimester, during their first diagnosis at maternity clinics in the Fourth Hospital of Hebei Medical University. The patients were 22-38 years of age and had suppressed menstruation 27-74 days. One hundred twenty-nine cases voluntarily accepted the thyroid function tests, had complete follow-up data, had no previous history of thyroid disease, had no significant surgical complications, and with confirmed single birth by B-ultrasound examination, were divided into two groups according to thyroid function, one was normal thyroid function group including 70 cases, who were 22-38 years of age, with suppressed menstruation for 29-74 days, and that were undergoing their first to third pregnancy. The other was hypothyroidism group including 59 cases, who were 22-30 years of age, with suppressed menstruation from 27-70 days, and that were undergoing their first to third pregnancy. Due to the fact that the age distribution of patients was non-normal, the Wilcoxon test was used for comparison and a $p > 0.05$ was judged as statistically significant.

Diagnostic criteria

The American Thyroid Association and the reference standard for diagnosis and treatment of thyroid disease guidelines recommend that the upper limit of serum thyroid stimulating hormone (TSH), in early pregnancy (less than 12 weeks of pregnancy), is 2.5 mU / l [5].

Specimen collection

Serum human chorionic gonadotropin (hCG), estradiol (E2), and progesterone (P) were measured in 164 patients, who were for the first time diagnosed in the present hospital, with threatened miscarriage in the first trimester, and included 129 patients with serum TSH, free triiodothyronine (T3) and free thyroxine (T4) monitored at the same time, which were sent to the clinical laboratory for quantitative analysis.

Therapeutic considerations

Serum hCG, E2 and P were measured in patients with threatened miscarriage, when serum P was above 25 ng/ml, threatened miscarriage was considered not caused by endocrine factors, and not treated with drugs. If serum P was between 10 and 30 ng/ml, progesterone was injected intramuscularly between 20 and 40 mg once a day, dydrogesterone was taken 10 mg twice a day, serum P and β-hCG were monitored once a week and B ultrasound was examined every two weeks. Progesterone was stopped when

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Table 1. — *The clinical data between normal group and hypothyroidism group.*

Groups	N. of cases	Age (mean ± SD	Pregnancies (n.)	Suppressed menstruation (days)
Normal group	70	27.66 ± 3.40	1 - 3	29 - 74
Hypothyroidism	59	25.66 ± 2.15	1 - 3	27 - 70

$p > 0.05$ has no statistical significance.

serum P was above 30 ng/ml, while dydrogesterone was continue taken as usual until 12 weeks of pregnancy. When serum P was below 10 ng/ml, it was considered that there was little significance for miscarriage, and outcomes were observed. Embryo development was observed by B ultrasound examination in all objects on a periodical inspection.

Therapy for hypothyroidism

In 129 patients, once hypothyroidism (including clinical hypothyroidism and subclinical hypothyroidism) was diagnosed, patients returned to the Hospital of Endocrinology as soon as possible and treated with LT4 as substitution, serum TSH, FT3, and FT4 were monitored every four weeks [6] and the levothyroxine dosage was adjusted accordingly.

Statistical methods

SPSS13.0 software was used, means were compared using the Wilcoxon rank sum test, P values were determined using receiver operating characteristic curve (ROC curve), the miscarriage rate between normal thyroid function group and hypothyroidism group which was treated with LT4 were compared using fourfold tables of the chi-square test and a $p < 0.05$ was judged as statistically significant.

Results

Among 164 cases of threatened miscarriage patients after treatment, 116 of them were tocolysed successfully, another 48 resulted in inevitable abortion, serum P levels were compared between the two groups and a $p < 0.01$ was judged as statistically significant (Table 2). Through ROC curve calculations, the group with a higher p value with higher sensitivity and specificity was selected as the predictive value to judge whether the fetal treatment was successfully or not in patients with threatened miscarriage, that is 14.74 ng/ml, with a sensitivity of 87.9% and a specificity of 72.9%.

According to the screening results of thyroid function, 129 patients were divided into two groups, of which 70 cases with normal thyroid function, whose progesterone levels of M (Q) was 18. 29 (12.28) ng/ml, distributed non-normal, other 59 hypothyroidism patients that had a normal P value distribution, whose P values (mean ± SD) were 22.18 ± 11.795 ng/ml.

P levels of the two groups were compared using the Wilcoxon rank sum test, the results of $p < 0.05$ confirmed that the P levels between the two groups had no distinct difference.

In the group of 129 cases with threatened miscarriage, there were 70 cases with normal thyroid function, including 59 cases of miscarriage and 46 cases of tocolytic failure. Among another 24 cases with hypothyroidism given sodium levothyroxine (LT4) substitution, 46 cases had miscarriages and 13 cases experienced tocolytic failure.

Table 2. — *Comparison of progesterone between miscarriage successful group and the inevitable abortion group*

Groups	N. of cases	Progesterone value
Miscarriage group	116	24.34 ± 9.56
Inevitable abortion group	48	10.95 ± 6.37

$p < 0.01$ has statistical significance

Table 3. — *Comparison of progesterone levels between normal thyroid function group and hypothyroid treatment group.*

Groups	N. of cases	Progesterone level (mean ± SD ng/ml
Normal thyroid function	70	18.29 (12.28)
Hypothyroidism with treatment	59	22.18 ± 11.795

Table 4. — *Comparison of the miscarriage rate between normal thyroid function group and hypothyroid treatment group.*

Groups	miscarriage successfully (cases)	Tocolytic failure (n.)
Normal thyroid function	46	24
Hypothyroidism with treatment	46	13

Fourfold tables of the chi-square test was used with SPSS13.0 software to carry out the result, that is: $x^2 = 2.349$ and $p = 0.125$, which is considered that the risk for miscarriage in patients diagnosed with hypothyroidism which LT4 substitution was similar to the level observed in the controls.

Discussion

The cause of threatened miscarriage is very complex, including endocrine diseases, autoimmune diseases, genetic abnormalities, and anatomic abnormalities et al. About 20% of threatened miscarriage is due to endocrine factors. P during pregnancy is very important to maintain normal pregnancy, which can affect the permeability of uterine smooth muscle to reduce the concentration of intracellular potassium, and increase the sodium ion concentration, resulting

in the relaxation of muscle fibers, the excitability decreasing, while reducing the sensitivity of pregnant uterus to oxytocin, and uterine contractions, so that zygote can grow and develop normally in the womb. For some reason, the low level of serum P values would lead to threatened abortion or miscarriage.

Serum P has been used as key indicators to judge the threatened abortion prognosis; this study also confirmed that serum progesterone levels play in a very important role in predicting threatened abortion pregnancy outcome. In this study, P value of 14.74 ng/ml was used as the predictive value to judge whether the treatment in patients with threatened miscarriage was successful or not. When serum P value was above 14.74 ng/ml before treatment, it may have led to a miscarriage, if the serum progesterone value was below 14.74 ng/ml, miscarriage was unlikely and its sensitivity and specificity was high. Positive treatment can be given to patients with threatened miscarriage who may have a good prognosis, those with poor prognosis should be offered an earlier termination of pregnancy earlier to avoid the meaningless miscarriage and over-treatment, or missed abortion caused by excessive miscarriage which may lead to secondary coagulation disorders, while avoiding the waste of medical resources and consuming energy and financial resources of the patients.

Pregnant women with hypothyroidism can lead to adverse pregnancy outcomes, such as spontaneous abortion, anemia, gestational hypertension, placental abruption, postpartum hemorrhage, preterm birth, low birth weight, neonatal respiratory distress syndrome, and fetal death miscarriage is one of the early complications [7,8]; however, the mechanism explaining the risk of miscarriage increased by thyroid disorders remains unclear. Currently, there are five hypotheses regarding the mechanism of this effect as following: The first theory states that the miscarriage risk appears to be not directly related to thyroid autoimmunity (TAI), but to immune imbalance [9]. According to the second theory, the increased risk of miscarriage should be attributed to the direct action of the thyroid autoantibody on the placenta, which has been validated in animal models [10-12], but still needs to be confirmed in human models. The third theory postulates that the increased risk of miscarriages is a result of a subtle deficiency in thyroid hormone concentrations due to a decreased adaptability of the thyroid gland to the increased demands of pregnancy, in the presence of TAI. With lower thyroid hormone levels and higher maternal TSH levels in pregnancy, the risk of miscarriage is increased. In a randomized controlled trial [13], the risk for miscarriage in patients with diagnosed TAI with LT4 substitution was significantly lower and similar to the levels observed in the healthy controls [14]. The fourth theory argued is that thyroid autoantibody positive rate is increases when women are older, since increased age is an independent risk factor for miscarriage [15], and TAI is attributed to the age factor. The last theory is corpus luteum hypothesis [16] which states that there are a variety of antibodies in the plasma of TAI, which could inhibit hCG action on its receptors, located in corpus luteum, by the immune cross-reactivity, this inhibition could cause luteal phase defect and lead to a decrease in steroid hormones production, such as P and estrogen, result in spontaneous miscarriages.

The results of this research support the third theory that the risk for miscarriage in patients with diagnosed hypothyroidism with LT4 substitution was significantly reduced and similar to the level observed in the healthy controls, and *p* between the two groups had no distinct difference, but not yet deny the corpus luteum hypothesis, to analyze the reasons: (1) the cause of spontaneous abortion is very complicated, mostly attributed to embryonic chromosomal factors, while the proportion of luteal phase defect caused by endocrine factors which is caused by hypothyroidism is very small, and could not rule out the interference of other factors; (2) in this study, considering economic conditions of patients, autoimmune thyroid antibody was not routinely estimated in patients who were in the group, and thyroid antibody was one of the independent risk factors increasing the abortion rate. The positive rate level of antibodies also affected the final result [17,18]; (3) the sample size was small and larger-scale clinical studies should be conducted to confirm the hypothesis.

At present, it has caused a high degree of attention in the medical profession that pregnant women with hypothyroidism lead to adverse pregnancy outcomes for both mother and child, and the incidence of hypothyroidism has increased each year, which is reported to be from 2% to 5% [19,20], and the thyroid antibody-positive rate reported is approximately 10% -15%. As early as 1999, the American Association of Clinical Endocrinologists proposed to screen TSH routinely for pregnant women and all planned pregnancies. Whether all pregnant women should have their TSH and thyroid antibody screened, is still controversial. Current evidence-based medicine does not yet support the screening of all pregnant women for thyroid function, but recommends to screen TSH in pregnant women with high risk for thyroid disease, such as the following: (1) hyperthyroidism, hypothyroidism, PPT, or partial hepatectomy, history of thyroid; (2) a family history of thyroid disease; (3) thyroid nodules; (4) thyroid-associated antibodies (known) positive; (5) hyperthyroidism or hypothyroidism symptoms or clinical signs, and accompanied with anemia, high cholesterol and low sodium aciduria; (6) diabetes type 1; (7) other autoimmune diseases; (8) infertile women should screen TSH as part of infertility associated with this; (9) head and neck history of radiation therapy; (10) miscarriage or preterm birth history [20].

Most scholars agree that patients with clinical or subclinical hypothyroidism once diagnosed should be treated with LT4 as substitution as early as possible, and variety of adverse pregnancy outcomes can be prevented and improved. Results of the research confirmed that the risk for

miscarriage in patients with diagnosed hypothyroidism in which LT4 substitution was similar to the level observed in the controls.

In summary, thyroid function screening and treatment has a very important clinical significance in early pregnancy. By strengthening thyroid function monitoring in high-risk patients and mission to get their coordination, early diagnosis and treatment would be done to improve the perinatal outcomes in both mother and child with hypothyroidism.

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Prediction of pregnancy outcomes with combined ultrasound scanning of yolk sacs and serum CA 125 determinations in early threatened abortion

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Summary

Objectives: To assess the predictive value of the combination of ultrasound scanning, yolk sacs and CA125 levels for pregnancy outcomes in early threatened abortion. *Materials and Methods:* A total 196 pregnant women at less than 12 weeks gestation were enrolled. They were assigned into: (A) normal pregnancy (n = 61); (B) early threatened abortion but with favorable outcomes after active treatment (n = 56); (C) pregnancy with spontaneous miscarriage and threatened abortions (n = 79). The yolk sacs were examined and serum CA125 levels were measured. *Results:* The visualization rate in groups A and B were significantly higher than that in group C. For the mean yolk sac diameter, there was a statistically significant difference between groups A and C ($p < 0.05$), B and C ($p < 0.05$), but no statistically significant differences were observed between A and B ($p > 0.05$). The mean serum CA125 levels were significantly different ($p < 0.05$) among three groups. The sensitivity, specificity, and Youden Index for predicting adverse outcomes using irregular shape, abnormal size, or non-visualization of the yolk sac were 81.01%, 85.71%, and 0.67, respectively. *Conclusion:* The combination of ultrasound scanning of yolk sacs and measurement of serum CA125 levels is of great value for predicting pregnancy outcomes.

Key words: Ultrasound scanning; Yolk sacs; CA125.

Introduction

Early threatened abortion is the most common complication in the first trimester of pregnancy; it presents as vaginal bleeding and/or cramping, which occurs in about a fifth of cases. About half of the women with early threatened abortion will be normal after positive clinical treatment, and the other half will have a miscarriage. Occasionally, bleeding may persist for weeks; thus, it becomes necessary to determine the possibility of continuing the pregnancy.

In the gestational sac, the earliest sonographic finding is the yolk sac, which can always be detected as a round anechoic area between the fifth and 12th week of pregnancy; rarely, it can be identified until the end of pregnancy.

The tumor marker CA 125, discovered using monoclonal antibodies against cells derived from the ovarian cancer cell line OVLA 433 [1], is present at high concentrations in human amniotic fluid throughout gestation [2]. Extracts of human decidua and chorion have been found to contain significant quantities of CA 125. In contrast, serum CA 125 levels are low in both maternal and fetal blood, and very little is found in the amnion and trophoblasts [3]. During the first trimester of pregnancy, serum CA 125 increases modestly, frequently reaching a peak value and then returning to its non-pregnancy range in late pregnancy.

The present study was performed to assess the value of the ultrasound scanning of yolk sacs and measuring CA125 levels, respectively, in predication of pregnancy outcomes of early threatened abortions. The authors then estimated the value of predicating pregnancy outcomes with the combination of the two indexes. The sensitivity and specificity were also calculated.

Materials and Methods

The present study was conducted in the First Affiliated Hospital of Henan University of Science and Technology. A total of 196 women in their first trimester of pregnancy were referred to the study center between October 2008 and May 2009; women with multiple pregnancies, impregnated by artificial insemination, with abnormal uterine development, and those with a history of smoking, hypertension, and diabetes were excluded. The study was conducted in accordance with the Declaration of Helsinki and approval by the People's Hospital of the First Affiliated Hospital of Henan University of Science and Technology. Informed written consent was obtained from all subjects. All pregnancies were examined through ultrasonography using a Voluson 730 real-time scan system, with a carrier frequency of 5-10 MHz for the vaginal probe. Gestational age was determined by ultrasonographic measurements of gestational sac diameter and fetal crown-rump length. The yolk sac diameter (YSD) was determined by placing the calipers on the inner limits of the longer diameter. Meanwhile, venous blood samples were collected from all pregnancies, which were centrifuged at 3,000 rpm and stored at –20°C. The CA125 levels were measured using commercial ReadyPack kits.

All pregnancies were followed for survival until 28 weeks by either a subsequent ultrasound scan or a telephone interview. According to the pregnancy outcomes, the women were divided into three groups. The first (A) group included 61 women with normal pregnancy, whereas the second (B) group was composed of 56 women who experienced early threatened abortion but with fa-

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vorable outcomes after active treatment. Meanwhile, 79 women whose respective pregnancies resulted in spontaneous miscarriage with threatened abortion were assigned into the third (C) group.

Statistical analysis was performed using SPSS 11.5 software. For continuous variables, the comparisons among groups were evaluated using analysis of variance (ANOVA). Determination of the significance of the differences between proportions for the count data was through a χ^2 test. *P*-values less than 0.05 were considered statistically significant.

Results

The mean age (ranging from 20 to 44 years) and mean gestational age (ranging from five to 12 weeks) of the three groups were calculated and they are shown in Table 1. There were no significant differences among the three groups ($p > 0.05$).

The yolk sac visualization rate in the three groups is shown in Table 2. The yolk sac was visualized in 56/61 cases (91.8%) in group A and 48/56 (85.71%) in group B, which are in contrast to 26/79 (32.91%) in group C. Therefore, significant differences were observed between groups A and C ($p < 0.05$) and between groups B and C ($p < 0.05$). No significant difference ($p = 0.381 > 0.05$) was observed between groups A and B.

The YSDs of women in the three groups are shown in Table 3. The mean diameter, maximum diameter, and minimum diameter of group A were 4.02, 6.10, and 2.86 mm, respectively; for group B, they were 3.72, 5.8, and 2.24 mm, respectively; and for group C, 5.34, 10, and 1.8 mm, respectively. Accordingly, a significant difference was observed between groups A and C ($p < 0.05$) and between groups B and C ($p < 0.05$), but there was no significant difference between groups A and B ($p > 0.05$). The mean YSD in the three groups by week are illustrated in Figure 1.

The serum CA125 levels of women in the three groups were measured and they are depicted in Table 4. The mean level, maximum level, and minimum level in group A were 17.38, 23.12, and 11.68 U/ml, respectively; in group B, 25.73, 60.74, and 17.57 U/ml; and in group C, 57.63, 84.47, and 45.64 U/ml, respectively. There was a significant difference among the three groups, and the trend of the serum CA125 in the three groups by week is presented in Figure 2.

The sensitivity, specificity, and Youden Index of the ultrasound scanning of the yolk sacs and the serum CA125 levels, and the combination of the two are shown in Table 5.

Table 1. — *The basic information of women among three groups.*

Group	N	Mean age (years)	Mean gestational age (weeks)
A	61	27.58 ± 3.3	7.8 ± 1.25
B	56	28.13 ± 3.5	7.9 ± 1.32
C	79	27.51 ± 3.2	8.2 ± 1.42

Table 2. — *The visualized rate of women in the three groups (%).*

Group	N	Visualized	Not visualized
A	61	56 (91.80)	5 (8.20)
B	56	48 (85.71)	8 (14.29)
C	79	26 (32.92)	53 (67.08)
Total	196	130 (66.32)	66 (33.68)

Table 3. — *The yolk sac diameter of women in three groups.*

Group	N	Mean diameter (mm)	Max diameter (mm)	Min diameter (mm)
A	61	4.02 ± 1.12	6.10	2.86
B	56	3.72 ± 1.23	5.80	2.24
C	79	5.34 ± 1.46	10.00	1.80

Table 4. — *The serum CA125 levels of women in three groups (U/ml).*

Group	N	Mean level (U/ml)	Max level (U/ml)	Min level (U/ml)
A	61	17.38 ± 6.02	23.12	11.68
B	56	25.73 ± 7.34	60.74	17.57
C	79	57.63 ± 9.08	84.47	45.64

Table 5. — *The predictive rate of three indexes' comparison.*

Item	Sensitivity (%)	Specificity (%)	Youden index
Yolk sac	81.01	85.71	0.67
CA125	91.14	83.93	0.75
Yolk sac+ CA125	98.32	71.94	0.70

Discussion

The present study is one of few to assess simultaneously the yolk sac through ultrasound scanning and the serum CA125 level during the first trimester of pregnancy.

Revolutionary technologic improvements and high-frequency transvaginal scanning have enabled the resolution of ultrasound imaging in the first trimester to increase, such that detailed early fetal development can now be well visualized [4]. Transabdominal ultrasound provides little information regarding the fetus before the eighth week of pregnancy. Transvaginal ultrasound can identify earlier the yolk sac, fetus, and embryonic cardiac activity, and can confirm intrauterine pregnancies at younger gestational ages [5]. In the present study, transvaginal ultrasound was performed in women at less than eight weeks gestation, and transabdominal or transvaginal ultrasound in women with more than eight weeks gestation in the trimester.

In a gestational sac, the earliest ultrasonographic finding is the yolk sac. A yolk sac with abnormal or irregular shape

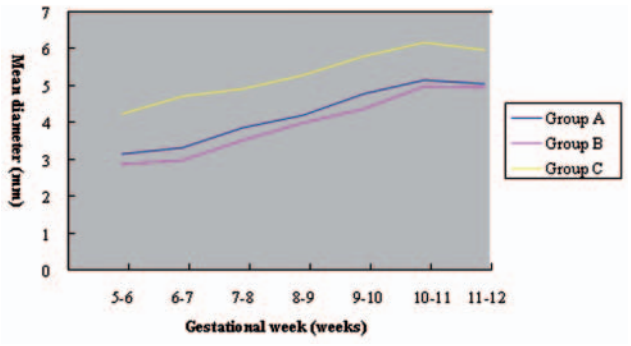

Figure 1. — The trend of the mean yolk sac diameter of women in three groups by week.

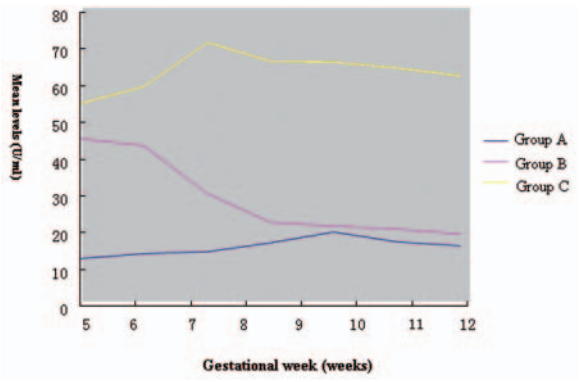

Figure 2. — The trend of mean serum CA125 levels of women in three groups by week.

that is not detected by transvaginal ultrasonography or is either too small or too large usually indicates adverse pregnancy outcomes.

The yolk sac is a round, sonolucent structure with a bright rim. However, transvaginal ultrasonography can be used to survey abnormally shaped yolk sacs, which generally results in poor outcomes [6]. In the present study, five women experienced adverse pregnancy outcomes because of abnormally shaped yolk sacs.

The yolk sac first appears during the fifth week of pregnancy. A yolk sac that is not visible under vaginal ultrasonography between five and ten complete weeks menstrual age is an indicator of a developmental disturbance during early pregnancy [7]. In the present study, the visualization rate in group A was 91.8% (in 56/61 cases), which is very close to the previous study, which indicates that with a reliable gestational age between five to ten weeks, the yolk sac is recognized in 158 of 172 normal pregnancies (91.9%) [7]. In group B, the yolk sac was visualized in 48/56 cases (85.71%). In contrast, the visualization rate was 32.92% (in 26/79 cases) in group C, which is in accordance with a previous study [7]. The five cases in group A and eight cases in group B wherein the yolk sacs were not visualized during ultrasound scanning resulted in favorable pregnancy outcomes based on the authors' follow up. However, all 53 women with non-visualized yolk sacs in group C ended in spontaneous abortion. Using statistical analysis, no significant difference was observed between groups A and B, but significant differences were observed between groups A and C ($p < 0.05$) and between groups B and C ($p < 0.05$).

Through ultrasound scanning, the YSD was determined by placing the calipers on the inner limits of the longer diameter. Normal-sized yolk sacs are usually indicative of favorable pregnancy outcomes, whereas abnormal-sized yolk sacs (too large or too small) are usually indicative of inevitable abortions. In the present study, in group A, the increase in YSD was found to be correlated with advancing gestational age, which is consistent with prior studies [8, 9]. Based on the results, 56 visualized yolk sacs had a mean diameter of 4.02 mm, which is in accordance with a previous finding that demonstrated that the mean YSD of normal pregnancy is within four to five mm [10]. Only one yolk sac with a diameter exceeding six mm was detected, which is quite close to the results of a study that stated that a yolk sac diameter exceeding six mm was observed in five of 253 normal pregnancies (2.0%) [7]. In group B, the mean diameter of 48 visible yolk sacs is 3.72 mm, which is slightly smaller than that in group A. However, statistical analysis revealed no significant difference between groups A and B ($p > 0.05$). In group C, among the 26 visualized cases, nine women who had YSDs exceeding six mm, which is in accordance with a previous statement, argued that YSDs above six mm can serve as an indicator of developmental disturbances in early pregnancy [7]. Up to 15 women with normal-sized yolk sacs and two women with small-sized yolk sacs had adverse pregnancy outcomes based on telephone follow up. The mean, maximum, and minimum diameters were 5.3, 10, and 1.8 mm, respectively. The mean diameter in group C was significantly larger than that in group A ($p < 0.05$).

Accordingly, the sensitivity, specificity, and Youden Index for predicting adverse outcomes using the ultrasound results of yolk sacs with irregular shapes, abnormal sizes, or non-visualized yolk sacs were 81.01%, 85.71%, and 0.67 respectively. The present results indicate that the early yolk sac measurement during gestation may be a useful marker for pregnancy outcomes [11].

In the present study, serum CA125 was also used to evaluate pregnancy outcomes. Serum CA 125 levels increase in early pregnancy and immediately after birth, thereby implicating the disintegration of the maternal decidua (i.e., blastocyst implantation and placental separation) as a possible source of the increase in tumor marker levels [12]. A significant increase in serum CA 125 levels was also reported in patients with vaginal bleeding and

impending spontaneous abortion [13, 14]. According to the present results, serum CA 125 levels remained low in group A, which slowly increased to a peak value and then dropped slowly to a certain low level. In group B, the serum CA125 levels were slightly higher from five to six gestational weeks, but decreased sharply from six to eight gestational weeks to levels during normal pregnancy. The values then declined slowly from eight to 11 gestational weeks to levels closer to normal pregnancy. In group C, the CA 125 levels slowly increase from five to seven gestational weeks. However, they increased sharply from seven to eight gestational weeks to a maximum value, and then decreased slowly to a rather higher level. The mean serum CA125 levels in the three groups were 17.38, 25.73, and 57.63 U/ml, respectively. There was a significant difference between groups A and C and between groups B and C. Therefore, the present findings coincide with those of a previous study, which demonstrates a highly significant increase in serum CA125 in women who aborted compared with those in the other two groups (the group with normal pregnancy outcomes and the group with women with threatened abortions but with continued pregnancies) [15].

The CA125 levels at 95% CI were calculated for group C, which has a lower limit of 55.57 U/ml. No woman was observed to have CA125 levels above 55.57 U/ml in group A. Meanwhile, nine women in group B with CA125 levels exceeding 55.57 U/ml resulted in favorable pregnancy outcomes after positive clinical treatment. When 55.57 U/ml was used as the cut-off value, the 72 women in group C with CA125 levels above the cut-off value and the nine women in group B had sensitivity, specificity, and Youden Index values of 91.14%, 83.93%, and 0.75, respectively. The present study shows that serum CA-125 measurement may be an inexpensive, easily available, sensitive, and specific predictor of outcome in threatened abortion, which results in loss [16].

In the present study, these two predictors combined together were also evaluated to have greatly improved sensitivity of up to 98.32%, specificity of 71.94%, and Youden Index of 0.70. The present findings are in line with a previous study [17].

In conclusion, the combination of ultrasound yolk sacs scanning and measurement of CA125 levels is of great value for predicting pregnancy outcomes, and may be an easy, cheap, and reliable way for predicting pregnancy outcomes for women with threatened abortion in the first trimester.

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The serum level of C-reactive protein in patients undergoing GnRH agonist protocols for in vitro fertilization cycle

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Summary

Background: The synchronization of the uterus and mature eggs at the molecular level is the key factor in embryo transfer, and the regulation of synchronization depends on a variety of cytokines. C-reactive protein (CRP), as the first acute phase reaction protein, is involved in the entire process of embryo transfer. The study is designed to investigate the correlation among CRP, sex hormone, controlled ovarian hyperstimulation (COH) cycle, and pregnancy outcome. *Materials and Methods:* Ninety-two patients who accepted in vitro fertilization (IVF) treatment cycles because of tubal factor were included in the study. Seventy treated cases were included to complete final analysis with the full set of results. Respectively on the second day of the menstruation (Day-2) in gonadotropin-releasing hormone agonist (GnRH-a) short program treatment, on the morning in human chorionic gonadotropin (hCG) treatment (Day-hCG) and the embryo transplant day (Day-ET), plasma CRP level was tested by enzyme-linked immunosorbent assay (ELISA). The correlativity among CRP level, sex hormone, COH, and pregnancy outcome was analyzed by statistical methods. *Results:* In the short program GnRH-a of 70 cases, there was no relationship between serum CRP level and the infertility age, gonadotropin (Gn) dosage, number of oocytes retrieved, the number of normal fertilization, and sex hormone. In the short program of GnRH-a, the change of serum CRP levels in Day-2, Day-hCG, Day-ET: serum CRP in Day-2 < Day-hCG < Day-ET and the level of serum CRP gradually increased in Day-2, Day-hCG, and Day-ET in both the pregnant group and non-pregnant group. In non-pregnant group, the ratio of hCG / D2 and ET / hCG-day were significantly higher than the pregnant group. The area under receiver operating characteristic (ROC) curve was 0.806, indicating the accuracy of diagnostic tests is medium. the authors chose the point which presents the ratio of CRP in Day-ET to Day-hCG which was less than 1.752 as a predictor of treatment outcome, the sensitivity of the experiment was 77.8%, and the specificity 75%. *Conclusion:* CRP as a sensitive inflammatory marker, CRP ratio of Day-ET/ Day-hCG could be a predictor of treatment outcome by ROC curve analysis in COH program.

Key words: C-reactive protein; Controlled ovarian hyperstimulation; GnRH; Embryo transfer.

Introduction

Human assisted reproductive technology (ART) is a developed new technology in recent decades, having greater prospects, which is the most effective treatment to infertility. The most representative method in ART is fertilization in vitro and embryo transfer (IVF-ET), also called test-tube baby. The success of embryo transfer is a key link in IVF-ET. After quality four- to eight-cell embryo was transplanted into patients' endometrium, whether the endometrium accepts the embryo has become the key to the growth of embryos. In the process of IVF-ET, controlled ovarian hyperstimulation (COH) is a potentially inflammatory reaction. whereas, the body's levels of inflammatory mediators might be the most important factor of the ability of endometrium to accept embryos [1]. Therefore, to explore levels of inflammatory factors in IVF-ET process is important to the success of IVF.

In the study of the inflammatory response, C-reactive protein (C of reactive protein, CRP), as the first acute phase reaction protein (ARP), participates in non-specific immunity and combines with a variety of pathogens and other polysaccharide substances. The complexes could activate the complementary system, neutrophils, mononuclear phagocytes, and the production of cytokines such as IL-6, IL-1, and result in the inflammatory response [2]. IVF test confirmed that the IL-1 could ensure normal pregnancy through the inhibition of uterine stromal cell decidualization [3]; IL-6 stimulates the production of protease and inhibin involved in the formation of the placenta, which is beneficial to the trophoblast and endometrial differentiation [4]. Therefore, the level of CRP in the body is closely related to the success of ET. Researches indicate that CRP could decrease the stability of nitric oxide synthase (nitric oxide synthase, NOS) mRNA in endothelial cell, and lead to reduced production of nitric oxide, endothelial cell apoptosis, thus inhibiting angiogenesis, as well as disturbing the implantation of the embryo [5]. Studies [1] found that CRP increases in IVF patients since the first week of oocytes retrieval in the future, and the CRP ratio of pregnancies between transplantation and oocyte retrieval day was lower than that of non-pregnancies, which has similar trend of IL-1 in Karagouni *et al.* study [6], indicating that the increase of CRP may facilitate the production of cytokines in IVF patients, changing the preimplantation endometrial environment, thus affecting embryo implantation. At the same time, as an inflammatory marker, there is no circadian fluctuation of CRP com-

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pared with other cytokines such as IL-1. Additionally, its high sensitivity, simple, and rapid clinical testing make it more economical than the LIF and IL-1 and receive more and more attention from scholars in the field of auxiliary reproductive study.

At present, the COH program is different between reproductive centers; the main difference lies in the gonadotropin-releasing hormone (gonadotropic releasing hormone of GnRH) analogues and their administration time [7]. GnRH analogues, include gonadotropin-releasing hormone agonist (GnRH-a) and gonadotropin releasing hormone antagonist (GnRH-anta). GnRH-anta rapidly inhibits excitatory effects of endogenous GnRH on the pituitary through the competitive combination of GnRH-R. While GnRH-a inhibits the release of gonadotropin (Gn) through down regulation of GnRH receptors (GnRH-R) and desensitization of pituitary Gn cells. This inhibition is a slow process, which could reduce the body's inflammation process and is preferred by researchers. In the present research, the authors detected the quantity of CRP in plasma at different time point, including the next day of GnRH-a short protocol ovarian hyperstimulation (COH) cycle period (Day 2), the day of hCG (Day-hCG), and the day of embryo transplant (Day-ET). They then explored its relationship between hormone quantities of plasma, COH, and other variables.

Materials and Methods

Ninety-two patients who accepted IVF treatment cycles because of tubal factor were included in the study. There were 22 cases of incomplete CRP results or hemolysis. Therefore, only the data of 70 women who had a full set of results were included to complete the final analysis, except for the patients subjected to intracytoplasmic sperm injection (ICSI), natural cycles, and frozen ET. Patients aged 25 to 38 years , normal basal body temperature, body mass index (BMI) < 30, six sex index (FSH, LH, PRL, E, P, and T) test was normal. The infection tests were negative for anti-sperm antibody, anti-endometrium. Before accepting IVF progesterone, the subjects did not receive Gn and clomiphene citrate therapy, and did not have previous serious systemic disease and ovarian hyperstimulation syndrome (OHSS). The short program of use GnRH-α: two days before menstruation, the subjects were given GnRH-a 0.1 mg by subcutaneous injection once every two days. The drug use time was adjusted according to B ultrasound monitoring of follicle diameter and menstrual cycle of patients. rFSH 225 IU once a day was added on the third day of menstruation. At the eighth day of menstruation, the growth of follicle was monitored by B ultrasound. Diphereline and Gonal-F were stopped, given hCG 10000IU by intramuscular injection at 9:00 pm, and harvested the oocyte after 36 hours when the following situations appeared: there were two or three dominant follicles whose diameter was 18~20 mm in vaginal, and the plasma E2 peak value was more than 2,000 pmol/l. At 48-72 hours after oocyte harvesting, one to two embryos were transferred intrauterine. Daily supplement progesterone 60 mg after ovulation was given. At 14 days after ET, urinary hCG was positive, and at six to eight weeks, clinical pregnancy characteristics, such as intrauterine gestation and fetal heart beat, were observed.

Table 1. — *In the short program of GnRH-a, the CRP level had no relationship with the infertility age, Gn dosage, number of oocytes retrieved, and fertilization rate ($\bar{x} \pm s$).*

Index	Serum CRP (mg/ml)		
	Mean ± SD ($\bar{x} \pm s$)	Pearson coefficient of correlation	*p* value
Serum CRP (mg/ml)	174.453 ± 136.597	–	–
Infertility age (years)	29.889 ± 3.984	0.101	0.691
Gn dosage (IU)	2044.286 ± 515.890	0.009	0.972
Oocytes retrieved (n)	13.741 ± 8.934	0.235	0.349
Normal fertilization Number (2pn) (n)	7.185 ± 5.241	0.115	0.648

Table 2. — *In the short program of GnRH-a, there was no relationship between the ratio of serum CRP/BMI, and E2 in Day-2, Day-hCG, and Day-ET ($\bar{x} \pm s$).*

	Day-2	Day-hCG	Day-ET
E2 (pg/ml)	56.584 ± 34.057	375.625 ± 1685.865	2783.952 ± 1962.871
Serum CRP/BMI	5.677 ± 2.481	6.847 ± 1.987	12.162 ± 4.982
Pearson coefficient of correlation	0.495	0.897	0.891
p value	0.505	0.103	0.109

Sample collection

Serum: respectively on the second day of menstruation (Day-2), on the day use of hCG (Day-hCG) early in the morning, on the transplant day early in the morning (Day-ET), five ml venous blood was drawn from each patient and centrifuged at 5,000 r/min for ten minutes. The upper serum was taken, then stored at -20°C. Serum samples were collected with the consent of the Clinical Ethics Committee and the informed consent of patients.

Specimens detection

The CRP in serum samples was detected by enzyme-linked immune sorbent assay (ELISA) method. The operation was in strict accordance with the kit specifications.

Statistical methods

The subjects were divided into the pregnant group and the non-pregnant group. Through the paired t-test, ANOVA analysis, and the classification and the regression trees (CART), the rate difference of transplantation/oocyte retrieval was determined. SPSS11.5 and CART were applied to perform statistical analysis; $p \leq 0.05$ was considered a significant difference.

Results

In the short program of GnRH-a in the 70 cases, there was no relationship between serum CRP level and the infertility age, Gn dosage, number of oocytes retrieved, and the number of normal fertilization, as shown in Table 1.

In the short program of GnRH-a, the relationship between CRP and sex hormone: as we all know, CRP and body weight have a significant correlation, and for infertility patients, their weight and sex hormones have a certain relationship. However, under the premise of no difference in the underlying sex hormone levels and BMI ≤ 30, there

Table 3. — *The comparison of CRP levels in Day-2, Day-hCG, and Day-ET ($\bar{x} \pm s$).*

	Serum CRP (mg/ml)
Day-2	120.351 ± 107.726**
Day-hCG	145.174 ± 97.245*
Day-ET	257.834 ± 160.745

* $p < 0.05$ compared with Day-ET; ** $p < 0.01$ compared with Day-ET.

Table 4. — *The clinical outcomes and infertility age in the short program of GnRH-a ($\bar{x} \pm s$).*

Clinical outcomes	Case No. (%)	Infertility age
Pregnancy	23 (32.86)	29.778 ± 4.265
Non-pregnancy	47 (67.14)	29.944 ± 3.963
Total	70 (100)	29.889 ± 3.984

was no correlation between serum CRP/BMI and E2, as shown in Table 2.

In the short program of GnRH-a, the change of serum CRP levels in Day-2, Day-hCG, Day-ET: serum CRP in Day-2 < Day-hCG < Day-ET. While the level of serum CRP gradually increased in the COH process. Single factor analysis of variance: the F and P value of serum CRP levels of different groups is 5.838 and 0.005, indicating that serum CRP concentrations in the groups had statistically significant differences. Therefore pairwise comparisons by the analysis of variance are shown in Table 3.

In the short program of GnRH-a, the comparison between the pregnancy and non-pregnant group: this study was aimed to understand the relationship between CRP and embryo implantation. Therefore clinical pregnancy was taken as the demarcation of the pregnant group and non-pregnant groups.

In the short program of GnRH-a, the clinical outcomes and infertility age is shown in Table 4. Regardless of pregnancy or non-pregnant group, the level of serum CRP gradually increased in Day-2, Day-hCG, and in day-ET. Moreover, there was no significant difference between the pregnancy and non-pregnant groups at the same time point. (respectively, *p* values were 0.366, 0.840, and 0.595). Therefore, the single factor analysis of variance to the concentration of the serum CRP at three different time points was performed: the F value was 2.625 in non-pregnant group, and *p* value was 0.096.There was no significant difference in serum CRP concentration between groups; pairwise comparisons also showed that there was no difference in different groups. The F value was 3.374 in pregnant groups, and *p* value was 0.052.There was no significant difference in serum CRP concentration between groups, but only the CRP concentration in Day-ET was higher than that of Day-2 ($p < 0.05$). The data are shown in Table 5 and in Figure 1.

The level of CRP in pregnancy and non-pregnant groups was parallel (Figure 1, Table 5). However, the ratios of CRP

Table 5. — *The comparison of CRP levels in Day-2, Day-hCG, and day-ET between pregnant and non-pregnant groups ($\bar{x} \pm s$).*

	CRP (mg/ml) in pregnant group	CRP (mg/ml) in non-pregnant group
Day-2	87.426 ± 66.035	145.103 ± 92.745
Day-hCG	144.828 ± 106.526	157.392 ± 136.309
Day-ET	243.742 ± 182.167*	280.809 ± 155.963*

* $p < 0.05$ compared with Day-2.

Table 6. — *In the short program of GnRH-a, the comparison of ratios of CRP level in Day-2 to Day-hCG, and Day-ET to Day-hCG in pregnant and non-pregnant groups ($\bar{x} \pm s$).*

	Day-hCG/Day-2	Day-ET/Day-hCG
Pregnancy	1.704 ± 0.991	1.474 ± 0.748*
Non-pregnancy	2.503 ± 0.535	2.566 ± 1.252
p value	0.634	0.043 ($p < 0.05$)

* $p < 0.05$ compare with the ratio of CRP in Day-ET to Day-hCG in non-pregnant group.

level in Day-2 to Day-hCG, and Day-ET to Day-hCG in pregnant and non-pregnant group had significant differences ($p < 0.05$), as shown in Table 6.

As shown in Figure 2, the area of ROC curve was 0.806, indicating the accuracy of diagnostic tests is medium. Then the point which presents the ratio of CRP was chosen in Day-ET to Day-hCG which was less than 1.752, as a predictor of treatment outcomes from the upper left corner of the figure. The sensitivity of the experiment was 77.8%, which signifies that the ratio of Day-ET to Day-hCG in 18 from 23 pregnant cases was less than 1.752. The specificity was 75%, which signifies that the ratio of Day-ET to Day-hCG in 36 from 47 pregnant cases was less than 1.752.

Discussion

Firstly, the authors identified that the serum CRP level has no relation with infertility age, Gn dosage, number of oocytes, and the number of normal fertilization in the stop GnRH-a protocols of COH and IVF-ET process. Furthermore, there was no correlation between the CRP/weight index ratio and E2. Secondly, in the stop GnRH-a protocols, the serum CRP level gradually increased in the COH process in both pregnant and non-pregnant groups. The level of serum CRP in D2 day (CRPD2) was less than that in the hCG day (CRPhCG) and the serum CRP level in hCG day (CRPhCG) was less than that in the ET day (CRP ET). However, at the same time point, there were no differences between pregnant and non-pregnant group. Thirdly, the ratio of CRP hCG/CRPD2 with CRPET/CRPhCG was significantly different between pregnant and non-pregnant groups. The sensitivity and specificity was 77.8% and 75% when using the ratio for predicting pregnancy.

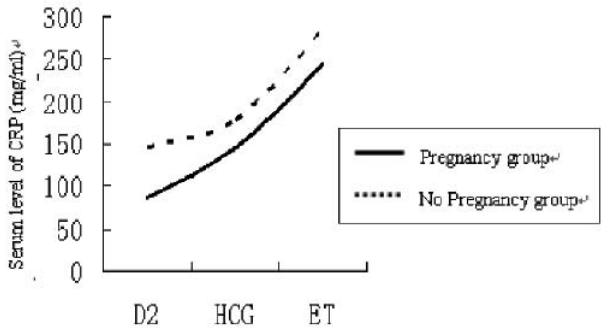

Figure 1. — The comparison of CRP level between pregnancy and non-pregnant groups $(\bar{x} \pm s)$.

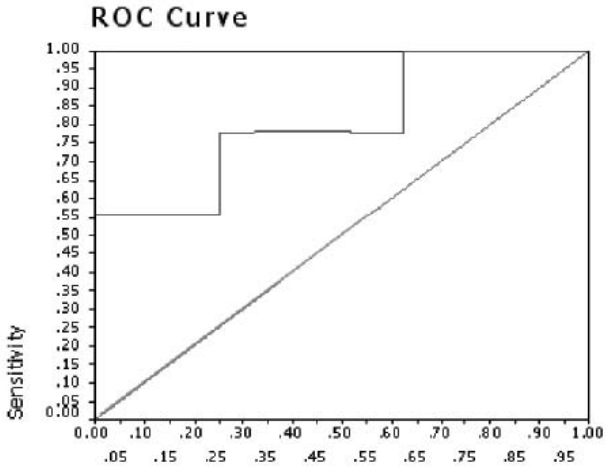

Figure 2. — The ROC curve in the short program of GnRH-a. The area under the curve is the area of ROC curve, diagonal line is the opportunity ROC curve.

CRP is a type of sensitive inflammation marker and was discovered in the serum of acute inflammation patient in 1930. There are no circadian fluctuations in plasma CRP concentration and the concentration is not affected by radiotherapy, chemotherapy, corticosteroids, and other treatment. The clinical detection can be conducted at any time of the day, therefore, CRP became a sensitive and reliable indicator [2, 8]. On one hand, CRP synthetized of by the liver participate in non-specific immune, combined with a variety of pathogens and other polysaccharide substances and with lecithin and nucleic acids in the presence of calcium. The combined complexes could activate the complement system as well as neutrophils, monocytes, and phagocytes with the production of cytokines as IL-1 and IL-6, triggering the opsonization and phagocytosis to invasion cells and showing inflammatory response. On the other hand, activated immune cells release a variety of cytokines as IL-6, can promote hepatocyte synthesis of CRP [2]. At present, the CRP could be detected in human serum, pleural effusion, and in follicular fluid. In the field of assisted reproduction, IL affect the reproductive activities in all aspects such as the regulation of egg development, maturity, ovulation, and other ovarian functions, as well as processes like fertilization, implantation, and pregnancy. In particular, IL-1 expression in embryonic and endometrial constitutes the material basis of the cross-talk between embryo and endometrium. IL-1 could induce and affect endometrial receptivity as well as the expression of LIF that mediates embryo implantation. Meanwhile, IL-6 increase instantaneously in the planting window and jointly promotes implantation with LIF [3, 4]. In addition, COH is an essential factor for the success of IVF-ET, with the most important complications as OHSS. Accurate OHSS is caused by large amount of cytokine increase or neutrophil activation in system infection [9-11]. So the authors found that the CRP is related with reproduction.

Current studies show that serum CRP level increases up from D2 day to hCG day and to ET day, which is consistent with the rising of E2 level in COH process. Additionally, the CRP level is stable at long-term without circadian disturbance. Therefore, even in the case of E2 declined due to loss of granule cells after egg retrieval, serum CRP is also increases. In an in vitro study of cytokines released from peripheral lymphocytes in super-ovulation patients, Orviet found that the level of interleukin cultured with whole blood significantly increased at E2 peaks in COH process [12]. Ricoux *et al.* also indicated that the increase of estradiol does not affect CRP level in the ovarian stimulation stage [13]. These researches support the results of this experiment and also proved the presence of infection in the COH and IVF-ET process. IL-1 can quickly produce CRP and preimplantation embryos in human can secrete IL-1. Therefore, IL-1 deletion will cause endometrial decidualization abnormalities and embryo resorption [13-15]. In this experiment, CRPhCG is higher than CRPD2 without significant difference and CRPET is significantly higher than CRPhCG. It can be inferred that the increase of CRP mainly commenced before implantation and after egg retrieval, which can be used as a signal of implantation. The initial adhesion process of the embryo and endometrium is regulated by their signal exchange and local factors promote embryo implantation through mechanisms of paracrine and autocrine.

The results also show that: the ratio of CRP (day of embryo transfer/day of hCG) is lower in pregnant cycle than that of non-pregnant cycle, which may indicate that the infection or potential infection level is lower in pregnant woman than that of non-pregnant women. In embryo implantation stage, in order of the embryo positioning and implantation, the endometrium will reduce the release of mediators of immunoregulation, as cytokines to stabilize lysosomal membrane to promote placental macrophages secreting prostaglandin E2, inhibit phospholipase C activity, block part of the prostaglandin synthesis, and inhibit

uterine contraction [16-18]. Furthermore, CRP could interact with both humoral infectious effect system and cellular infectious effect system [19-20]. Therefore, fundamentally speaking, there appears to be some immunosuppressive effect to the proper maturity of endometrium and embryo from CRP, which indicates that the infection degree of endometrium plays a role in the establishment of a suitable endometrial receptivity. For clinical, it is important to find an appropriate cutoff for CRP ratio to predict the success or failure of early embryo implantation. Inflammatory state and degree of IVF-ET patients could be understood in real time through the detection of indicators, the level of which could be used as guidance for individualized fetus protection and duration of anti-inflammatory treatment. In this experiment, through the ROC curve analysis of the COH program, the authors calculated the CRP ratio on transplant day and hCG day as < 1.752, which was used as a predictor of treatment outcome. However, the sensitivity and specificity of the predictor is not satisfactory and a more appropriate predictor could be established by increasing the number of detected cases, joint detection from multi-center, and expanding the sample size, conditions permitting.

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Application of two-dimensional echocardiography combined with enhanced flow in diagnosing fetal heart malformation

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Summary

Objective: The current study aims to evaluate the diagnostic accuracy of two-dimensional echocardiography combined with enhanced flow (e-flow) imaging for fetal heart malformation. *Materials and Methods:* A total of 1,639 pregnant women were enrolled. They were examined using fetal echocardiography combined e-flow. The obtained results were compared with those by postnatal examination or post-induction autopsy. *Results:* Complete data were obtained from 1,286 out of the 1,639 fetuses (78.46%). Two-dimensional echocardiography combined with e-flow imaging had sensitivity, specificity, a misdiagnosis rate, and a missed diagnosis rate of 98.0%, 99.3%, 2.0%, and 0.7%, respectively. It has a consistency evaluation Kappa value of 0.970 ($p = 0.000$). *Conclusion:* Two-dimensional echocardiography combined with e-flow is an accurate and reliable diagnostic method for fetal heart malformation. It has high sensitivity and specificity.

Key words: Fetal echocardiography; Congenital heart disease; Enhanced flow imaging.

Introduction

Fetal heart malformation is one of the congenital diseases seriously influencing fetal intrauterine development and neonatal survival; it has an incidence of 6‰ to 8‰ in live neonates [1]. Fetal echocardiography is a feasible, effective diagnostic method for heart malformations. It is of great significance for fetal intrauterine detection and intervention therapy, neonatal monitoring and corrective surgery, and perinatal neonatal mortality reduction. The diagnosis of fetal heart malformation is particularly important for the detection of the fine structure in fetuses during first- and second-trimester pregnancy. Although traditional color Doppler imaging can demonstrate the pulmonary venous blood flow and rate in third-trimester fetuses, its limitations in sensitivity and resolution influence its display of the pulmonary venous blood flow in first- and second-trimester fetuses. The pulmonary venous blood flow rate in first- and second-trimester fetuses is rather slow because of a quite thin vascular inner diameter. Meanwhile, the existence of channels, such as the foramen ovale, ductus arteriosus, and ductus venosus at fetal stage that maintains the fetal blood circulation system stable while keeping the pressures on the right and left heart systems equivalent to each other. This factor also entails difficulty in diagnosing fetal blood shunting to some degree.

Enhanced flow (e-flow) imaging is a technique which can clearly display low-speed blood signals; it enhances temporal and spatial resolutions by differentiating blood signals from tissue signals [2].

In the current study, two-dimensional echocardiography combined with e-flow imaging was used for fetal heart malformation screening. The accuracy of this method in diagnosing fetal heart malformation was then assessed.

Materials and Methods

Subjects

A total of 1,639 pregnant women that received fetal echocardiography at Henan Provincial Hospital and Beijing Anzhen Hospital were involved. Their ages ranged from 17 to 45 years with an average of 28.54 ± 4.41 years. Their gestational ages ranged from 16 to 40 weeks with an average of 26.36 ± 3.95 weeks. Subjects with complete data of prenatal echocardiography, postnatal echocardiography and related examination or surgery, autopsy after induction of labor, and so on were selected, whereas those with incomplete data were excluded.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Beijing Anzhen Hospital and Henan Provincial Hospital. Written informed consent was obtained from all the participants.

Guidelines and standards for screening

Fetal heart malformation was screened according to the guidelines and standards for fetal echocardiography recommended by the American Society of Echocardiography [3]. The standard views included the abdominal transverse plane, four-chamber view, left outflow tract plane, right outflow tract plane, short axis of both cardiac ventricles, three vessels and trachea view, long axis of the aortic arch, and long axis of the ductus arteriosus arch.

Case collection

Normal fetal echocardiograms: fetal echocardiography and physical examination were performed within one to 90 days after childbirth. The results were compared with those echocardiograms.

Abnormal fetal echocardiograms: pregnancy continued. Fetal echocardiography and physical examination were performed

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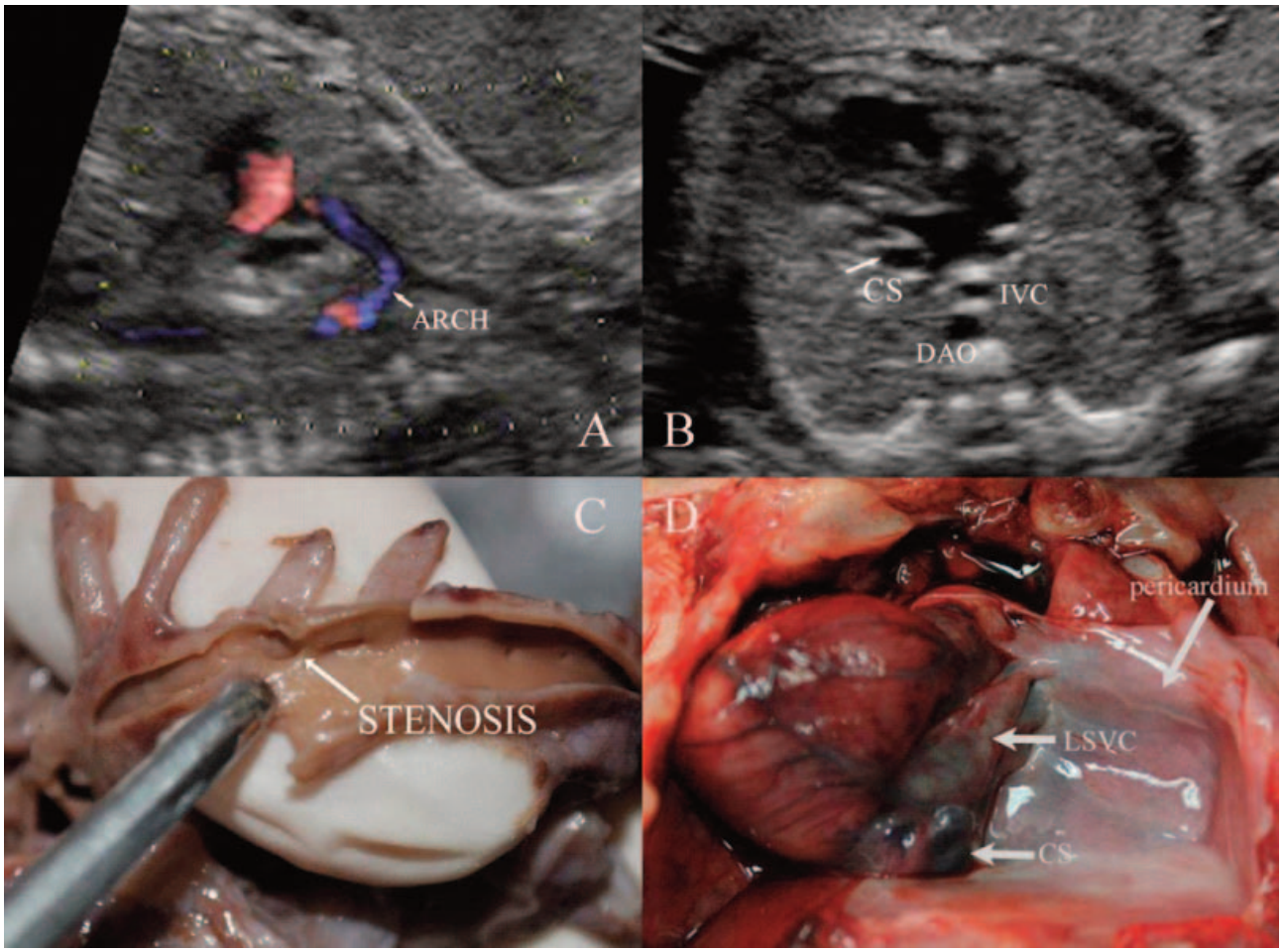

Figure 1. — Comparison between the echocardiography diagnosis (coarctation of the aortic arch, permanent left superior vena cava, and coronary sinus dilation) at the gestational age of 25 weeks and the autopsy pathology after induction in the same fetus. A: The plane of the long axis of the aortic arch shows a thin diameter at the aortic arch (the coarctation of the aortic arch is indicated by the arrow), but color Doppler examination does not show noticeably abnormal blood flow signals; B: the four chamber plane shows a widened coronary sinus in the left atrioventricular groove; C: post-induction autopsy shows a ridge between the subclavian artery and the left common carotid artery at the aortic arch which leads to diameter narrowing (the narrowed site is indicated by the arrow); and D: post-induction autopsy shows a downward connection between the permanent left superior vena cava and the coronary venous sinus outside of the left atrium and ventricle. ARCH: the aortic arch; DAO: the descending aorta; IVC: the inferior vena cava; LSVC: permanent left superior vena cava; and CS: the coronary venous sinus.

within 1 d to 90 days after childbirth. The results were compared with those echocardiograms. For the neonates subjected to cardiac surgical correction, pre-, intra-, and post-operative related data were collected and then compared with those echocardiograms.

Abnormal fetal echocardiograms: for the women whose pregnancy was terminated due to severe fetal heart malformation, the results of fetal autopsy were compared with those echocardiograms.

Statistical analysis

Data were analyzed using SPSS16.0 software. The diagnostic effectiveness of two-dimensional echocardiography combined with e-flow imaging for fetal heart malformation was evaluated using diagnostic test, and its diagnostic consistency for fetal heart malformation was evaluated using Kappa test.

Results

Clinical data

The pregnant women aged from 17 to 45 years with an average of 28.54 ± 4.41 years. Their gestational ages ranged from 16 to 40 weeks with an average of 26.36 ± 3.95 weeks. Complete data were obtained from 1,286 (78.46%) out of the 1,639 women and 353 (21.54%) were lost to follow-up.

The effectiveness evaluation

The diagnostic effectiveness evaluation results of two-dimensional echocardiography combined with e-flow imaging for fetal heart malformation are summarized in Table 1. Compared with the standard method, two-dimensional

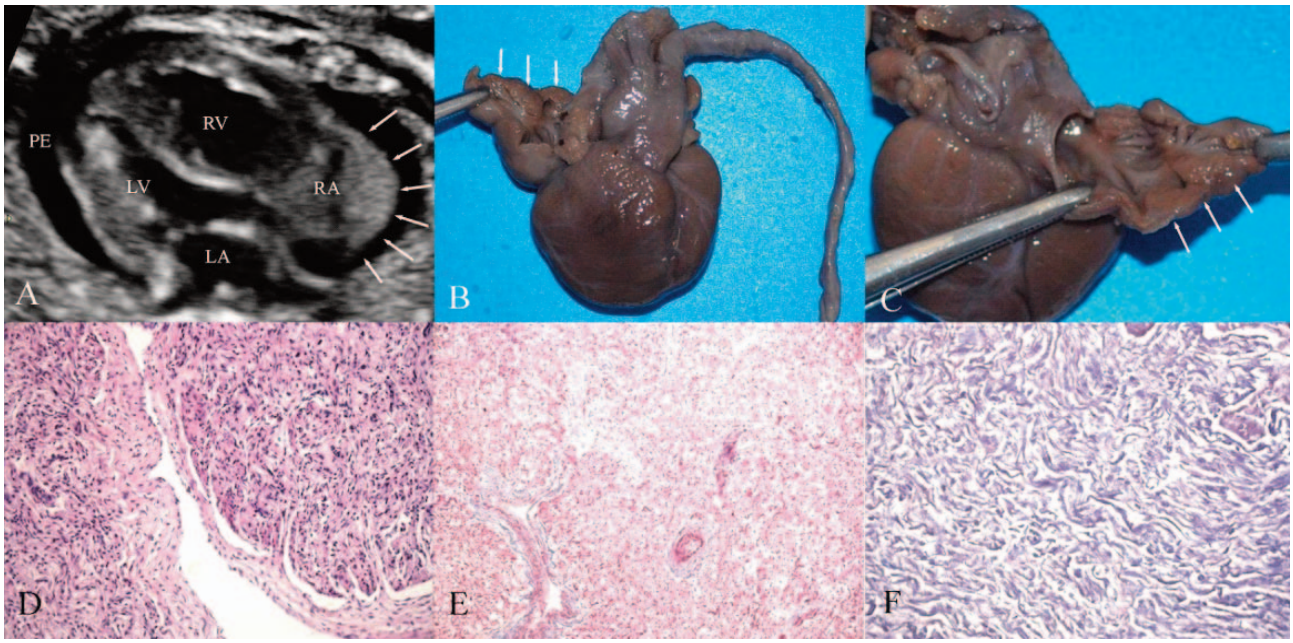

Figure 2. — Comparison between the echocardiography diagnostic results (right atrial aneurysm) at the gestational age of 26 weeks and the general and microscopic views after post-induction autopsy in the same fetus. A: The four chamber plane shows a thickened right atrial wall (indicated by the arrow) with a thickness of 3.8 mm and a small amount of hydrops in the pericardial cavity; B and C: The autopsy shows a thickened right atrial wall (indicated by the arrow); D: HE staining (×200) shows hyperplastic cells on the atrial wall in bunchy arrnangement with some deranged cells and vessels of different sizes distributed in the interstitia; E: PTAH staining (× 100) shows that the cells are striated muscle tissue cells; and F: Masson staining (× 100) shows that the cells are striated muscle tissue cells.

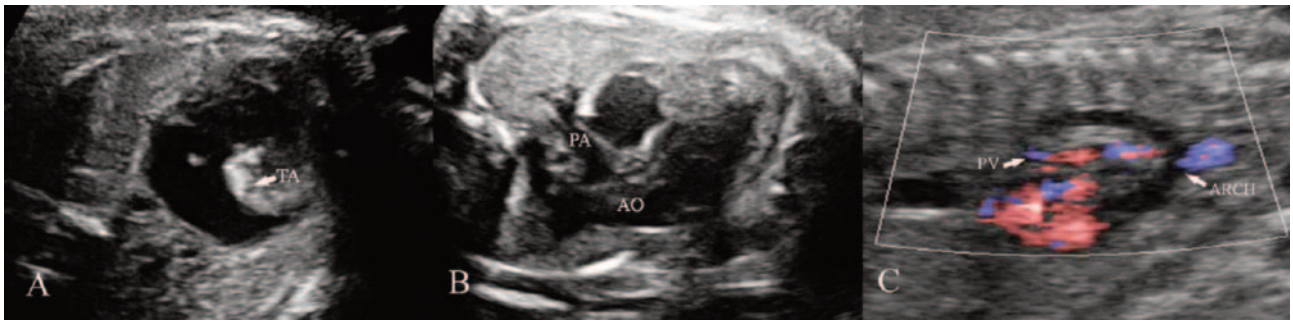

Figure 3. — Fetal echocardiography diagnostic results of one fetus at the gestational age of 29 weeks (total anomalous pulmonary venous drainage (supracardiac type), functional single atrium (right heart auricle isomerism), right atrioventricular valvular closure, single ventricle, pulmonary artery atresia, and right aortic arch complicated with vascular ring formation). A: The four chamber plane does not show high-level echoes other than one muscular strong echo from the tricuspid valve area (the tricuspid valvular closure is indicated by the arrow); B: the five chamber plane shows the aorta located on the right side of the pulmonary artery (right transposition of the great arteries) and the closure at the pulmonary artery opening; and C: The plane of the long axis of the aortic arch shows the abouchement of the pulmonary veins into the superior vena cava. TA: tricuspid atresia; AO: the aorta; PA: the pulmonary artery; PV: pulmonary veins; and ARCH: the aortic arch.

echocardiography combined with e-flow imaging had sensitivity of 98.0%, specificity of 99.3%, a misdiagnosis rate of 2.0%, a missed diagnosis rate of 0.7%, a total coincidence of 99.1%, a Youden index of 97.3%, a positive predictive value of 97.9%, and a negative predictive value of 99.3%.

The respective true positive, false negative, and false positive cases before childbirth, as well as during follow-ups by two-dimensional echocardiography combined with e-flow imaging are summarized in Tables 2, 3, and 4. Two hundred and thirty-nine cases (18.6%) were true positive, 1035 (80.5%) were true negative, seven (0.5%) were false negative, and five (0.4%) were false positive. Among the true positive cases, one manifested coarctation of the aortic arch, permanent left superior vena cava, and coronary sinus dilation (Figure 1), and one by right atrial aneurysm (Figure 2). The false negative cases presented with common pulmonary vein atresia (Figures 3 and 4).

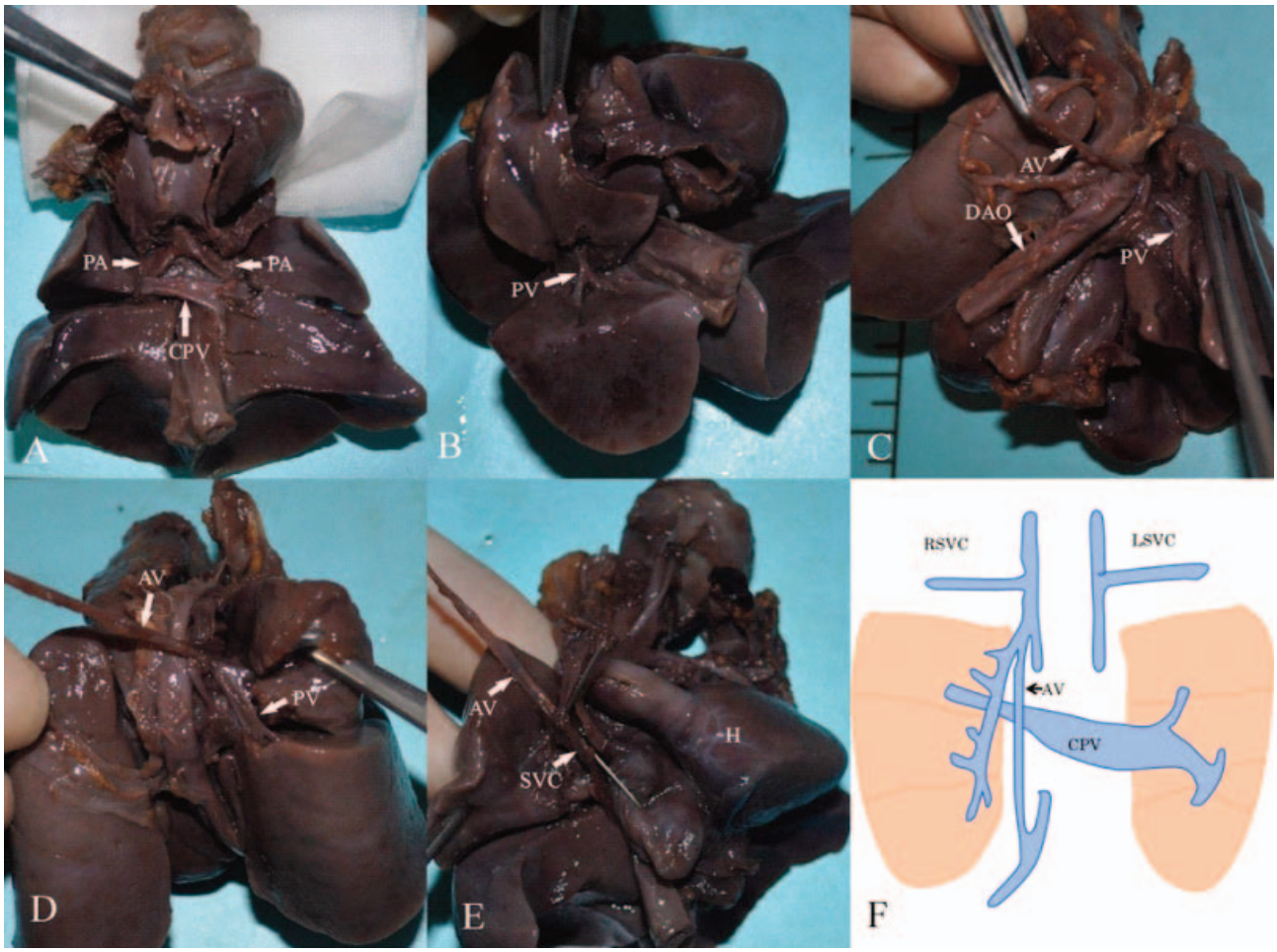

Figure 4. — The autopsy results of the same fetus in Figure 3 (partial common pulmonary vein atresia (atresia of the common cavity of the left superior, left inferior, and right superior pulmonary veins), supracardiac partial anomalous pulmonary venous drainage (the right inferior, middle, and superior branches converge into the azygos vein and then into the superior vena cava), permanent left superior vena cava (innominate venous absence), unroofed coronary sinus syndrome (total type), single atrium, tricuspid atresia, single ventricle, pulmonary artery atresia, right aortic arch, aortopulmonary collateral formation, and ductus arteriosus absence). A is the anterior view: the heart upturns, and the left superior, left inferior, and right superior pulmonary veins converge to form a common cavity which has no connection with the heart; B, C, D, and E, respectively, display the convergence of the right inferior, middle, and superior pulmonary veins into the azygos vein and then into the superior vena cava; and F is the view of partial common pulmonary vein atresia and supracardiac partial anomalous pulmonary vein drainage. CPV: common pulmonary vein atresia; PA: the pulmonary artery; PV: pulmonary veins; AV: the azygos vein; DAO: the descending aorta; SVC: the superior vena cava; H: the heart; RSVC: the right superior vena cava; and LSVC: the left superior vena cava.

Table 1. — *Comparison between two-dimensional echocardiography combined with e-flow imaging and follow-up examination in diagnosing fetal heart malformation (cases).*

Methods		Postnatal recheck, operation, and autopsy after induction Positive	Negative	Total
E-flow	Positive	239	5	244
	Negative	7	1035	1042
	Total	246	1040	1286

The consistency evaluation

The consistency between two-dimensional echocardiography combined with e-flow imaging and the standard method in diagnosing fetal heart malformation is summarized in Table 1. The consistent Kappa value was 0.970 ($p = 0.000$) which indicates that two-dimensional echocardiography combined with e-flow imaging had good consistency with the standard method in fetal heart malformation diagnosis.

Discussion

Fetal heart malformation is the most common congenital disease that seriously influences fetal intrauterine development and neonatal survival [4]. Fetal echocardiography is a technique which was first applied and whose clinical value was confirmed by Winsberg in 1972 [5]. Since then, M-mode

Table 2. — *True positive cases of fetal heart malformation diagnosed by two-dimensional echocardiography combined with e-flow imaging and their follow-up examination.*

Case types	Case number	Follow-up examination		
		Postnatal echocardiography	Postnatal operation	Autopsy
Ventricular septal defects	45	39	6	-
Total endocardial cushion defects	21	5	-	16
Single ventricle	15	2	-	13
Hypoplastic left heart	10	-	-	10
Hypoplastic right heart	7	2	-	5
Aortic stenosis	13	3		10
Pulmonary artery stenosis	15	5	2	8
Pulmonary atresia with intact ventricular septum	2	–	–	2
Ebstein's anomaly	8	–	–	8
Fallot tetrad	12	5	2	5
Persistent truncus arteriosus	15	2	–	13
Complete transposition of great arteries	10	–	–	10
Anomalous pulmonary vein drainage	10	2	–	8
Double outlet right ventricle	20	5	–	15
Ductus arteriosus early shrinkage	15	15	–	–
Heart neoplasms	11	1	–	10
Vascular ring	7	5	–	2
Valvular mucinous degeneration	2	–	–	2
Inferior vena cava interruption	1	–	–	1
Total	239	91	10	138

Table 3. — *False negative cases of fetal heart malformation diagnosed by two-dimensional echocardiography combined with e-flow imaging and their follow-up examinations.*

Fetal echocardiography	Follow-up examination
Total anomalous pulmonary vein drainage (intracardiac type) and permanent left superior vena cava	Autopsy: Severe hypoplastic right lung and permanent left superior vena cava
Total anomalous pulmonary vein drainage (supracardiac type), total endocardial cushion defects, persistent truncus arteriosus, left pulmonary artery stenosis, and permanent left superior vena cava	Autopsy: common pulmonary vein atresia (total type), total endocardial cushion defects, persistent truncus arteriosus, left pulmonary artery branch stenosis, and permanent left superior vena cava
Total anomalous pulmonary vein drainage (intracardiac type), single atrium (right auricle isomerism), tricuspid atresia, single ventricle, pulmonary artery atresia, and complicated pulmonary artery ostial stenosis	Autopsy: partial common pulmonary vein atresia, partial anomalous pulmonary vein drainage (supracardiac), single atrium, tricuspid atresia, single ventricle, pulmonary artery atresia, and aortopulmonary collateral formation
Pulmonary valve stenosis, pulmonary artery trunk and branch aneurysmal dilatation, ventricular septal defect, and tricuspid regurgitation	Autopsy: Pulmonary valve defects, pulmonary artery trunk and branch aneurysmal dilatation, and ventricular septal defect
Left ventricle glare points	Postnatal echocardiography: mild pulmonary valve stenosis
Left ventricle glare points	Postnatal echocardiography: mild pulmonary valve stenosis
Normal	Postnatal echocardiography: ventricular septal defects complicated with membranous aneurysm formation and mild tricuspid regurgitation

two-dimensional spectral Doppler, color Doppler, tissue Doppler, harmonic wave power Doppler, spatio-temporal image correlation, high-resolution blood flow imaging, two-dimensional gray scale imaging of blood flow, and three-dimensional imaging have emerged; these techniques all play an important role in evaluating fetal heart structure, blood circulation, and heart function [6-12].

E-flow employs the broadband reception technique and adds motion artifact suppression to coherent imaging. It successfully differentiates the color signal zone and the two-dimensional zone from each other. This differentiation enhances sensitivity; meanwhile, it avoids the problem of blood flow signal spillover during traditional color Doppler imaging, which radically improves temporal and spatial resolutions to enable the fine blood circulation to be reflected and thus to effectively prevent blood flow signal spillover under high sensitivity. In this study, the application of the e-flow technique to fetal echocardiography demonstrates that it improves the resolution of the display of the micro-vessels and low-velocity blood flows and reduces the spillover of color blood flow signals in fetal pulmonary vein and ductus venosus imaging.

Table 4. — *Five false positive cases of fetal heart malformation diagnosed by two-dimensional echocardiography combined with e-flow imaging and their follow-up examination.*

Two-dimensional echocardiography	Follow-up examination
Ebstein abnormalities, tricuspid regurgitation (severe), pulmonary valve absence, ventricular septal defect (membranous)	Autopsy: Ebstein abnormalities, no pulmonary valve absence, and no ventricular septal defect
Aortic dysplasia and ventricular septal defect	Autopsy: aortic root stenosis without ventricular septal defect
Ventricular septal defect (membranous)	Postnatal echocardiography: normal
Ventricular septal defect (peri-membranous)	Postnatal echocardiography: normal
Ventricular septal defect (muscular)	Postnatal echocardiography: normal

Forbus *et al.* evaluated the diagnostic accuracy of two-dimensional color Doppler for fetal heart malformation and obtained an accuracy rate of 89.5% [13]. They further classified misdiagnosed and missed cases: the missed diagnosis or misdiagnosis rates of single ventricle deformity, septal defects, valvular deformity, and conotruncal defects were 5%, 8%, 11%, and 25%, respectively. Only prenatal four-chamber views are not sufficient with some heart abnormalities [14]. Extra scanning of the left and right outflow tracts can improve the detection rate of fetal heart malformation [15-17]. However, even after this extra strategy is applied based on four-chamber view, the missed diagnoses of some fetal heart malformations can still occur. Based on the aforementioned, to increase the correct diagnosis rate for fetal heart malformation and to reduce the missed diagnosis and misdiagnosis rates, the e-flow technique was applied based on the planes in the guidelines and standards for fetal echocardiography recommended by the American Society of Echocardiography in this study.

The screening of 1,286 cases of fetal heart malformation in this study shows that the e-flow technique can demonstrate fetal low-velocity blood flow such as that in the pulmonary veins, foramen ovale, aortopulmonary collaterals, ductus venosus, and therefore has a great advantage in the diagnosis of pulmonary venous lesions, small collateral flow in persistent truncus arteriosus, interatrial shunt right before foramen ovale atresia, interruption of the inferior vena cava, vascular ring, and so on. However, there were still false positive and false negative cases in this study. Reasons for this phenomenon were analyzed as follows:

1) *A lack of the knowledge of rare heart malformations:* In this study, two cases with total anomalous pulmonary venous drainage according to prenatal diagnosis were confirmed with common pulmonary vein atresia by post-induction autopsy. Common pulmonary vein atresia is a type of rare heart malformation which often leads to neonatal death shortly after birth if not corrected timely [18]. Lucas *et al* first reported three neonates with common pulmonary vein atresia in 1962 and according to them, the deaths of the three neonates occurred at 3, 22, and 28 days after birth, respectively [19]. Deshpande *et al* detected three cases of common pulmonary vein atresia out of 1,326 collected congenital heart disease samples [20]. In common pulmonary vein atresia, the formed common cavity lies at the posterior wall of the left atrium. Thus, the blood flow signal in this common pulmonary vein is likely to be erroneously identified connected to the heart atrium or the systemic veins by ultrasonography and consequently, the malformation is misdiagnosed as anomalous pulmonary venous drainage. Therefore, under such a condition, the pulmonary veins should be carefully observed to ensure whether they are connected with the atrium or not;

2) *Influence of the special structure and hemodynamic properties at fetal stage:* In this study, two cases with normal prenatal pulmonary valvular flow rate were observed with mild pulmonary valvular stenosis after birth. The possible reason may be as follows: most blood flow signals from the inferior vena cava flow into the left heart atrium via the foramen ovale, and a small part flow into the right ventricle and pulmonary artery through the right atrium; during fetal period, lungs are unexpanded and do not communicate with the external environment, and the pulmonary vascular bed presents high resistance; with the closure of the foramen ovale after birth, the majority blood that originally enters into the left atrium from the inferior vena cava via the foramen ovale completely flows into the right ventricle and pulmonary artery rather than shunt; meanwhile, as the neonate develops, and the blood volume increases, and so does the blood flow entering into the pulmonary artery via the pulmonary valve. Because of these structural and hemodynamic changes, the phenomenon that a normal flow rate at the pulmonary valve orifice during fetal period turns into an increased flow rate at the same site is likely to occur;

3) *Influence of extracardiac malformation on the intracardiac structure:* In this study, one case with anomalous pulmonary venous drainage according to the prenatal diagnosis was confirmed with severe right lung dysplasia by the post-induction autopsy. The possible reason was analyzed: Unilateral lung dysplasia can lead to pulmonary venous unilateral absence; the left atrium can only receive unilateral pulmonary venous blood backflow; this condition results in an increase in the diameter of the left atrium rather than anomalous pulmonary venous drainage. Therefore, when fetal echocardiography is performed for fetal

heart malformation screening, to increase its diagnostic accuracy, secondary cardiac structure changes caused by extracardiac organ lesions, apart from intracardiac structure abnormalities, should be carefully observed.

To summarize, two-dimensional echocardiography combined with the e-flow technique is an effective, reliable diagnostic method for fetal heart malformation andhas high specificity and sensitivity; furthermore, the application of the e-flow technique on the recommended planes in the standard diagnostic method can reduce missed diagnosis and misdiagnosis of fetal heart malformation and increase diagnostic accuracy. However, a larger sample size is required to further summarize and analyze the causes for false and negative positivity, increase ultrasonic diagnostic skills, and provide a more reliable basis for the application of the new ultrasound technique to the diagnosis of fetal heart malformation.

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Case Reports

Complete eradication of chronic long standing eczema and keratosis pilaris following treatment with dextroamphetamine sulfate

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Summary

Purpose: To present two other dermatologic conditions related to a disorder of sympathetic nervous system hypofunction common in women that respond to treatment with dextroamphetamine sulfate – chronic eczema and keratosis pilaris. *Materials and Methods:* Case 1 was a patient with chronic eczema of 30 years duration was started on treatment for other conditions related to the sympathetic neural hyperalgesia edema syndrome, i.e., migraine headaches and chronic fatigue syndrome. Case 2 who also had chronic eczema also had a skin condition frequently associated with eczema – keratosis pilaris and he was started on dextroamphetamine sulfate for chronic fatigue syndrome. *Results:* Not only did the headaches and chronic fatigue syndrome in both patients markedly improve following sympathomimetic amine therapy but so did the eczema and keratosis pilaris. *Conclusions:* Eczema and keratosis pilaris are two more chronic dermatologic conditions besides chronic urticaria and prurigo nodularis that respond extremely well to treatment with dextroamphetamine sulfate. Case 2 shows this condition is not restricted to females.

Key words: Chronic eczema; Keratosis pilaris; Sympathomimetic amines; Sympathetic nervous system hypofunction; Sympathetic neural hyperalgesia edema syndrome.

Introduction

Atopic eczema is a chronic relapsing inflammatory skin condition related, at least in part, to defects in skin barrier function [1]. This defect leads to the classic eczematous skin lesions which may respond to topical glucocorticoids and topical calcineurin inhibitors, e.g., tacrolimus or pimecrolimus [1]. Sometimes microbial colonization and superinfection are implicated in exacerbations which respond to topical or systemic antimicrobial treatment [1].

Severe cases may be treated with systemic anti-inflammatory therapy including systemic glucocorticoids, cyclosporine A or mycophenolate mofetil [1]. New experimental methods undergoing clinical trials includes cytokine and chemokine therapy, e.g., anti tumor necrosis factor alpha drugs [1].

There is evidence that the sympathetic nervous system acts to reduce inflammation possibly by inhibiting cellular permeability [2]. Diminished sympathetic nervous activity has been found in patients with chronic pruritic skin disorders [3]. Some skin disorders, e.g., chronic urticaria refractory to standard therapy, have been found to respond to treatment with sympathomimetic amines [4, 5].

The present case report describes the complete remission of eczema of 30 years duration following treatment with the sympathomimetic amine dextroamphetamine sulfate. Furthermore not only did the eczema improve but one of the cases also had marked improvement of chronic keratosis pilaris of many years duration.

Case Report

Case 1

A 47-year-old woman presented with severe and frequent migraine headaches refractory to standard therapy. She was advised that a condition exists where diminished activity of the sympathetic nervous system leads to a variety of pathological conditions that respond quickly and efficiently to therapy with sympathomimetic amines [6]. These conditions include severe migraines resistant to standard therapy, and have been found to respond to sympathomimetic amine treatment [7, 8].

A trial of dextroamphetamine sulfate extended release capsules 15 mg twice daily was initiated with subsequent decrease in the intensity and frequency of the migraines. After two months on this therapy she relayed to the authors that an interesting phenomenon occurred; her eczema of 30 years duration had completely disappeared. She questioned whether this may be related to her dextroamphetamine sulfate therapy.

The patient was first diagnosed with eczema at age 18, which began with intensely pruritic lesions below the right elbow with

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spread to the contralateral elbow and subsequently to the entire length of both arms. Intermittently she would develop lesions in other areas of the body, including her eyelids and knees; however, these lesions would only last two months whereas the upper extremity lesions were persistent.

The only treatment that the patient received was betamethasone cream on an alternating three months on and three months off schedule, which provided only minimal relief. After 12 years she discontinued all therapy and resolved to just persevere with the condition. Following only two months of therapy with dextroamphetamine sulfate, the lesions had completely cleared up. The total remission has lasted two years thus far. While she continued the dextroamphetamine sulfate there have been no untoward side effects from the medication.

Case 2

A 45-year-old male presented with chronic fatigue syndrome. He was aware of the authors' treatment with dextroamphetamine sulfate for this condition and sought therapy [9, 10]. He demonstrated marked improvement in his chronic fatigue syndrome on 60 mg dextroamphetamine sulfate extended release capsules. He also noted interestingly that not only did his eczema of 30 years duration almost completely disappear but he had marked improvement of his keratosis pilaris which is a common skin condition in which the protein keratin forms hard plugs in the hair follicles forming small skin colored papules with the hair follicle. This condition is frequently associated with atopic dermatitis (eczema). The patient stated that this is the only time in 30 years he has had an improvement in the acne or the keratosis pilaris and for the first time he "did not have chicken skin".

Discussion

Though a spontaneous remission of the eczema in this woman is possible, the 30-year length of the eczema without any previous remission makes it likely that the sympathomimetic amine treatment was responsible for the benefit. The authors have found similar success in the treatment of long-standing severe urticaria lending credence that the benefit in this patient was not unique and that this therapy is likely to have benefit in other dermatologic pruritus disorders [4, 5, 11]. The authors have now treated over 20 cases of chronic severe urticaria resistant to standard therapy that have responded quickly and effectively to treatment with dextroamphetamine sulfate, and in fact, to date there has not been one failure.

The authors have also seen marked improvement in a severe case of prurigo nodularis that had failed to respond to antihistamine, intralesional glucocorticoids, phototherapy, and thalidomide (unreported at this time). The proposed mechanism of action of sympathomimetic amine therapy is that the drug corrects sympathetic nervous system hypofunction leading to a correction of a cellular permeability defect. The prevention of the absorption of toxins, chemicals, and bacteria eliminates the stimulus which would otherwise initiate an inflammatory reaction. There is evidence that diminished sympathetic nervous system activity in the skin plays an important role in the pathophysiology of disorders of chronic pruritus, including, but not limited to, prurigo nodularis [3].

It is unclear why in given individuals only certain tissues are affected, and why the same defect in the sympathetic nervous system can lead to such a wide degree of varying manifestations, particularly in women. These manifestations are often chronic, treatment-resistant disorders and include, but are not limited to, edema, weight gain, headaches, arthritis, fibromyalgia, chronic fatigue syndrome, pelvic pain, interstitial cystitis, esophageal motility disorders, gastroparesis, pseudointestinal obstruction, Crohn's disease, severe constipation, pseudopheochromocytoma, and vasomotor instability [2, 6, 12]. All of the above mentioned conditions have also been reported to respond very well to treatment with dextroamphetamine sulfate when all other types of treatments have failed.

Dextroamphetamine sulfate in the dosage prescribed (usually no more than 30 mg per day) is extremely well tolerated with no risk of addiction or withdrawal symptoms at these levels. The authors report this case with the hope that it will stimulate interest so that other clinicians will try this therapy before proceeding with riskier treatments, e.g., systemic anti-inflammatory agents [1]. It is also hoped that this case report may spark interest in a larger controlled trial for sympathomimetic amines in the therapy of chronic treatment-resistant eczema and other chronic pruritic disorders of the skin.

The condition now referred to as the sympathetic neural hyperalgesia edema syndrome is predominantly a disorder found in women. It is responsible for most pelvic pain conditions and the authors usually prescribe dextroamphetamine sulfate as the second line therapy if oral contraceptives are not sufficient [13]. They prefer sympathomimetic amine therapy over surgical intervention [14].

Case 2 was presented not only to substantiate by a second case that sympathomimetic amine therapy can markedly improve long standing chronic eczema and to report improvement of a new entity keratosis pilaris, but to also remind the reader that the defect of sympathetic nervous system hypofunction can also be seen in males.

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Pregnancy management in Behçet's disease treated with uninterrupted infliximab. Report of a case with fetal growth restriction and mini-review of the literature

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Summary

Background: The mutual impact of Behçet's disease (BD) and pregnancy is variable and still unclear. Among the safe drugs administered, the newer infliximab (IFX) was rarely experienced in pregnancy, particularly in the third trimester. *Case:* The authors report a pregnancy with fetal growth restriction at 36 weeks in a 31-year-old primigravida with symptomatic BD, treated with uninterrupted monthly IFX and daily enoxaparin. The patient was induced at 38 weeks and had an uneventful vaginal delivery of a healthy baby. The postpartum period and following six months were uneventful for mother in terms of BD exacerbation, and newborn in terms of potential risks of neonatal BD and/or infections due to late immunosuppressive IFX administration. *Conclusion:* Because of the inconstant mutual impact, BD pregnancies should be precautionary considered at "potential high-risk" and need a careful and close monitoring by a multidisciplinary team with specific expertise.

Key words: Behçet's disease; Vasculitis; Infliximab; Pregnancy; Obstetric outcome; Fetal growth restriction.

Introduction

Behçet's disease (BD) is a relapsing multisystemic vasculitis first described in 1937 as a distinct clinical entity characterized by the clinical triplet: oral aphthous ulcers, genital ulcers, uveitis [1]. Since then, additional features such as arthritis, thrombophlebitis, erythema nodosum, gastrointestinal lesions, central nervous system lesions, vascular injuries, and hypercoagulability have been included in the variable pattern of the disease [2].

Although the unknown etiology of BD, autoimmune and microbial origin have been suggested in terms of an autoimmune reaction (HLA-B5 and HLA-DR5 alloantigens) set off by infectious agents such as Herpes Simplex Virus 1 or Streptococcus species in genetically predisposed individuals [3].

Behçet's disease begins in the third decade and occurs endemically in the Mediterranean regions, Middle East, and Far East: highest prevalence in Turkey (80-370 cases per 100,000); middle prevalence in Japan, Korea, China, Iran, and Saudi Arabia (13.5 - 20 cases per 100,000); low prevalence in Western countries (0.12 - 0.33 per 100,000 in the USA) [3].

The incidence of BD in childbearing age suggests a careful management of maternal course and obstetric outcome in pregnant patients, however, the mutual influence of BD and pregnancy is variable and still unclear. Generally, BD tends toward remission during pregnancy, but the overall risk of poor obstetric outcome could be relatively increased. Both the largest and the most recent reviews of the literature reported an improvement of BD in up to 60% of pregnant women and about 30% of relapses of various severity, with rare cases of maternal venous thrombosis. Concerning obstetric outcome, the same two reviews showed 9-30% of fetal losses, with less than 5% of intrauterine growth retardation (IUGR) and preterm birth [4, 5].

Among the safe drugs commonly used to treat BD in pregnancy (corticosteroids, colchicine, cyclosporine, azathioprine), the newer infliximab (IFX) appear to be safe in the first two trimesters [6], but very rare experiences for uninterrupted treatments up to third trimester are reported [7, 8].

Due to increasing awareness of the risks of pregnancy in BD, the authors report a case of a pregnancy with late fetal growth restriction in a 31-year-old woman with symptomatic disease treated with uninterrupted IFX. Moreover, a mini-review of the literature on the maternal effects and the obstetric outcome in BD pregnant women is performed. Finally, the safety of IFX treatment in pregnancy is briefly reviewed and discussed.

Case Report

In 2009, a diagnosis of BD was made in a 27-year-old woman for an episode of oral ulceration plus left uveitis and positive pathergy test, according to the International Study Group criteria for the diagnosis of BD: recurrent oral ulceration plus at least two of recurrent genital ulceration, eye lesions, skin lesions, or positive pathergy test [9]. Furthermore, a transient ischemic right pyramidal syndrome with aphasia was contextually detected. Therefore, a BD therapy with monthly intravenous IFX 5 mg/kg was instituted and a remission of the disease was achieved.

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In 2012, the 31-year-old case-patient (eumenorrheic, gravida 0) spontaneously conceived (singleton), with uninterrupted maintenance of monthly IFX therapy and start of daily subcutaneous enoxaparin 4,000 IU. The pregnancy, carefully monitored in the present hospital outpatient by both obstetrician and rheumatologist, uneventfully coursed for mother and fetus until the hospitalization at 36 weeks plus two days of anamnestic gestational age for maternal vagal syncope symptoms, paresthesia on face and upper extremity, and episodic obscuring of vision (neurological consult: no focal signs of central nervous system involvement and negative EEG; cardiologic consult: no cardiocirculatory alterations and negative ECG). Fetal surveillance by ultrasound revealed a fetus on cephalic presentation with growth restriction of more than two weeks (abdominal circumference 290 mm), but a normal and normally inserted placenta (Grade 2), normal amniotic fluid (Amniotic Fluid Index 12.1), and normal pulsatility index (PI) of umbilical artery at Doppler study (0.89). Maternal blood samples showed mild anemia (hemoglobin 9.4 g/dl) and moderate neutrophilia (neutrophils 13,020 / mm^3), with normal values of biochemical tests, including clotting profile, erythrocytes sedimentation rate (ESR), and C-reactive protein (CRP).

The pregnant was closely monitored during the following two weeks, until the ultrasonographic suggestion, at 38 weeks plus four days, of stopped fetal growth (abdominal circumference 304 mm), with decrease of amniotic fluid (Amniotic Fluid Index 9.9) and umbilical PI (0.80). Therefore, the patient was induced and had an uneventful vaginal delivery of female healthy baby weighing 2,400 kg (Apgar score 9-10 at 1-5 minutes, pH 7.32, pCO_2 41.0 mmHg). A normal placenta was detected and a second-degree vaginal tear was sutured without pathergy-like inflammatory reaction around the site and/or wound healing alterations.

Postpartum period was uneventful, and mother and newborn were discharged at three days from delivery. Afterwards, patient continued to be monitored closely for eight weeks for the risk of BD flares, and heparin was given for additional four weeks for thromboembolic prophylaxis. At present six-month follow-up, the mother continues IFX therapy and does not present BD exacerbation, and the infant is normal without any sign of BD and/or infections.

Discussion

BD is a heterogeneous vasculitis with a broad spectrum of clinical presentations, and its reciprocal influence with pregnancy is relatively variable between patients and even during different pregnancies in the same patient. These controversial outcomes during pregnancy could reflect the protean nature of BD and the different ethnic study groups, but also the pregnancy-induced immunosuppression, also explaining the remissions during gestation [10]. Generally, pregnancy does not have a deleterious effect on the course of BD and may improve it, however, BD may adversely affect pregnancy with a variably increased rate of miscarriage and IUGR, as reported in the present case.

In particular, in the largest case-control study (31 BD patients, 77 pregnancies), remissions were significantly more frequent during both pregnancy and postpartum periods (70.1% and 61.0%, respectively), while exacerbations were observed only in 15.6% and 16.9%, respectively ($p < 0.001$) [2]. Rather similar conclusions were achieved by three different case series (sum: 48 BD patients, 115 pregnancies) reporting approximately half entered remission during pregnancy, whereas a third-quarter of patients experienced disease flares [11-13]. Moreover, these data were confirmed by the largest (131 BD patients, 229 pregnancies) and the most recent (published on February 2013) reviews of the literature, respectively: improvement of BD in up to 60% of pregnant women (mostly limited to non-severe cases), about 30% of relapses of various severity (especially around delivery) [4, 5]. On the other hand, two series (sum: 43 BD pregnancies) reported the opposite effect and 56-66% of their patients worsened during pregnancy, whereas 33-44% improved [10,14]. Anecdotally maternal thromboembolic events as cerebral venous thrombosis [15], superior vena cava thrombosis and pulmonary embolism [16], fatal colonic perforation [17], inferior vena cava and suprahepatic venous thrombosis (Budd-Chiari syndrome) in the puerperium were described [12].

Concerning obstetric outcome, in the aforementioned large case-control study, pregnancy complications (26.2% vs. 1.9%, $p < 0.001$) and miscarriage (20.8% vs. 6.6%, $p = 0.020$) rates were significantly higher in the study group, but not significantly higher gestational hypertension (3.8% vs. 0.3%) [2]. However, a large series (59 BD patients, 144 pregnancies) did not report an increased risk of pregnancy complications compared with 20 healthy pregnant women [12]. The two aforementioned reviews of the literature showed 9-30% of fetal losses (9.2% in the systematic review), with less than 5% of IUGR and preterm birth (0.8% and 1.3%, respectively, in the systematic review) [4, 5]. Finally, neonatal outcome was good and did not differ from the controls [2, 12]. Minor and transient neonatal disease (commonly cutaneous and/or oral aphthous lesions), probably caused by transplacental passage of maternal antibodies, were rarely described only in symptomatic mothers [18-20].

Among the drugs commonly used to treat BD [21], only corticosteroids, colchicine, cyclosporine, and azathioprine are considered safe at conception and throughout pregnancy [22]. Recently, newer agents such as the anti-TNF-alpha monoclonal antibody IFX, also used in the present case also, have been used to treat other inflammatory conditions in pregnancy and appear to be safe. Data on more than 300 pregnancy showed that IFX carries low fetal risk during conception and the first two trimesters, and suggest to consider discontinuation in the early third trimester in order to minimize late fetal exposure for the risk of neonatal immunosuppression [6]. Nevertheless, in a small retrospective series on inflammatory bowel disease, IFX treatment during pregnancy revealed to be safe for the mother and the fetus for uninterrupted treatments also [7]. To date, the present authors found only a very recent paper in the literature reporting a case of safe and successful treatment with repeated IFX 5 mg/kg in a woman diagnosed with BD at 12 weeks of pregnancy, with improvement of all symptoms and normal full-term delivery [8]. In the present authors' opinion, both the effective and safe use of IFX in BD pregnancy up to third trimester, therefore, render this present case report particularly interesting.

In this case, a 31-year-old primigravida with asymptomatic BD uninterruptedly treated with monthly IFX had an uneventful pregnancy, until 36 weeks of gestation when maternal disorders and fetal IUGR were detected, as sometimes reported in the literature [4,5]. Labor was induced at 38 weeks of gestation with an uneventful vaginal delivery of female healthy baby weighing 2,400 kg (caesarean section was not considered for the absence of any genital ulceration). A normal placenta was detected, without any sign of necrotizing villitis and/or decidual vasculitis, in fact rarely reported in the literature [23]. The vaginal tear did not present pathergy-like inflammatory reaction and/or wound healing alterations, as sometimes reported as a result of excessive action of white blood cells mimicking the signs of infection.

On the contrary of other reports, maternal clotting profile, ESR, and CRP were normal, although the pathogenesis of thromboembolic events in BD patients is still unclear, and conflicting results about the role of thrombophilic parameters such as protein C, protein S, antiphospholipid antibodies, and factor V Leiden have been reported [24]. Nevertheless, thromboembolic prophylaxis with daily subcutaneous low-molecular-weight heparin was started at conception, given throughout pregnancy, and continued for four weeks after delivery, as sometimes recommended in women with previous ischemic thrombosis.

The postpartum period and the closely monitored follow-up six months were uneventful for mother, considering the risk of flares shortly after delivery [25], and for the newborn, considering the potential risks of neonatal BD [18-20] and/or infections due to IFX late immunosuppression [6].

Conclusions

BD is a very heterogeneous syndrome and, similarly, the reciprocal impact with pregnancy is variously reported: in most cases, pregnancy does not have a deleterious effect on the clinical course of BD, however, the disease can adversely affect the obstetric outcome, with increased risk of fetal loss and IUGR. Because of these unclear and inconstant course of gestation and disease, these pregnancies should be considered at "potential high-risk" and, therefore, they require the knowledge of the possible reciprocal impacts, careful planning, and close monitoring by obstetricians, rheumatologists, and internists with specific expertise.

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Dichorionic twin pregnancy discordant for anencephaly: two cases with different management

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Summary

Background: Prevalence of anencephaly in dichorionic twins is higher than in singleton pregnancies. The authors report two cases with two different management strategies. *Case 1:* Spontaneous dichorionic diamniotic twin pregnancy with the second twin diagnosed with anencephaly at 12 weeks gestation. Selective feticide was performed at the age of 13.2 weeks. Vaginal delivery occurred at 39 weeks, and birth weight was 2,850 g. *Case 2:* Dichorionic diamniotic twin pregnancy discordant for anencephaly in the second twin was diagnosed at 13 weeks gestation. An expectant management was decided. Preterm delivery occurred at 35 weeks due to hydramnios of the affected fetus, delivering a healthy newborn weighing 2,300 g and an anencephalic neonate who died immediately after delivery. *Conclusion:* Anencephaly should be diagnosed as soon as possible, idealistically at 11-13+6 weeks ultrasound (US) scan, in order to offer the most appropriate counselling to the parents, ranging from selective feticide or expectant management. This short series suggests that selective early feticide may increase gestational age and birth weight.

Key words: Anencephaly; Twin pregnancy; Prenatal diagnosis; Selective feticide; Expectant management.

Introduction

Anencephaly is a neural tube defect incompatible with life. Intrauterine death rate is about 25% and postnatal survival is usually less than 48 hours in these cases [1]. The incidence rates of anterior neural tube defects, anencephaly and encephalocele, are increased among twins compared with singletons [1-3]. Also, congenital anomalies that include neural tube defects are increased in monochorionic twins in comparison to dichorionic twins [1, 4].

During the first-trimester scan, many major fetal abnormalities, including anencephaly, can be reliably diagnosed [5]. In twin pregnancies discordant for anencephaly, the two main risks for the unaffected co-twin arise from either the spontaneous death of the anencephalic fetus or the development of polyhydramnios [1, 4, 6]. The diagnosis of such anomaly in the first trimester of gestation allows the health professional to establish the best strategy to minimize the risks, especially death or early premature delivery, of the normal twin. In dichorionic twin pregnancies discordant for anencephaly, the two management options are selective feticide (SF) by intracardiac injection of potassium chloride (KCl) or an expectant management with serial ultrasound (US) examinations [1, 6].

Counselling parents in this situation may be a difficult task. A first series suggested that the best management for these pregnancies was the expectant management with serial US in order to minimize the risks of miscarriage that may occur after SF. If polyhydramnios is detected amniodrainage or late selective feticide could be offered. However, this conclusion was based on the presence of only one case of miscarriage in a series of nine cases managed with conservative management. In addition, in cases managed with conservative management, 57% developed polyhydramnios and, in total, 20% had late invasive procedures (amniodrainage or late SF) [6]. Risks of these procedures were not completely evaluated. On the contrary, in a further series and systematic review [7, 8], recommendation of SF was performed on the basis of a longer duration of gestation in this group without significant increased perinatal mortality.

Therefore counselling to these parents has not been completely resolved. This is an important issue, not only considering the health problem, but also the psychological impact of carrying an anencephalic fetus along gestation with the uncertain risk about how this condition may affect the healthy baby. On the other hand, moral and religious issues should be obviously respected.

The aim of the present study was to report a short series of two cases occurring in the same year, and therefore counselled in the same way but managed differently after knowing the parents' decision. A discussion about the risk and benefits of the two managements is presented. The authors describe the outcomes of both pregnancies and discuss the benefits and risks of each one of them. The authors aimed to know which one of these two managements could better improve the outcome of the normal twin.

Case Reports

Case 1

A 38-year-old primiparous woman with a spontaneous twin pregnancy attended this hospital for a 12 week scan. Abdominal two-dimensional (2D) US revealed a dichorionic diamni-

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otic twin pregnancy with the second twin affected with anencephaly. The parents received counselling and decided to have a SF. The procedure was conducted with intracardiac injection of 1.5 cc of KCl at 13.2 weeks. Follow-up US were performed every two weeks, showing normal fetal growth, anatomy, and amniotic fluid volume. The maternal hematological and coagulation parameters remained normal. Vaginal delivery occurred spontaneously at 39 weeks. Birth weight was 2,850 g and Apgar scores at one and five minutes were 7 and 9, respectively, requiring resuscitation type I.

Case 2

A 39-year-old primiparous woman with a twin pregnancy conceived with in vitro fertilization (IVF) was controlled in this hospital. At 13 weeks gestation the abdominal 2D US revealed an anencephaly in the second twin. On this occasion, the parents decided not to perform SF, but an expectant management. US monitoring showed intrauterine growth restriction and moderate hydramnios in the affected twin. A magnetic resonance imaging (MRI) scan was performed at 28 weeks gestation in order to rule out central nervous system abnormalities in the healthy twin. Spontaneous preterm labour occurred at 34.4 weeks and the patient was hospitalized. A single course of steroids for lung maturation was given. Cesarean section was performed at 35 weeks gestational age due to breech presentation of the first twin. The first newborn weighed 2,300 grams, and Apgar scores at one and five minutes were 6 and 8, respectively. No congenital abnormalities were observed and he was admitted in the neonatal intensive care unit due to low weight. He was discharged without any complication at the third day after birth. The second twin weighted 1,640 grams and his Apgar scores at one and five minutes were 1 and 0, respectively, No anomalies other than anencephaly were observed.

Discussion

Neural tube defects, such as anencephaly, are increased in twin pregnancies compared with singletons [1-3]. It is not clear if the cause of this phenomenon could be attributed to the twinning or to the mode of conception. In a recent study of 43 pregnancies diagnosed with anencephaly, Ben-Ami *et al.* concluded that twin pregnancies conceived by assisted reproductive technology constituted a high-risk group for anencephaly OR = 24.6 (CI = 11.4-53.2). It is a possible synergistic effect of both twinning and assisted reproductive technology [2].

An early diagnosis of dichorionic twin pregnancies discordant for anencephaly between 11+0 to 13+6 weeks gestation should be performed to offer the parents management options of the pregnancy: expectant or SF [6].

There is controversy regarding which treatment is best, expectant management or SF. Data from dichorionic twin pregnancies discordant for anencephaly managed expectantly demonstrated that although the rate of either miscarriage or fetal death between 12 and 23 weeks is similar to that in dichorionic pregnancies with normal fetuses (about 1%) [6, 7], the rate of early preterm delivery is increased, probably associated with a high risk of polyhydramnios [1, 6, 8]. In these cases, polyhydramnios is caused by an impairment of the swallowing reflex of the anencephalic fetus and the management in severe cases may include amniodrainage or late SF. Invasive management in late pregnancy has potential complications, such as rupture of membranes, chorioamnionitis or fetal death [6, 8, 9]. Vandercruys *et al.* observed, in a group of 35 dichorionic twin pregnancies discordant for anencephaly with expectant management, an incidence of 55% of polyhydramnios between 25 and 31 weeks gestation, requiring invasive procedures in 35% of them, which represents 20% of the total [6]. In the present case, the patient managed expectantly developed a moderate hydramnios and delivered at 35 weeks.

SF in dichorionic twins is performed by US-guided fetal intracardiac injection of KCl in the second trimester. Although there is no consensus regarding which is the best gestational age to perform the SF, most series recommend performing it as early as possible in order to reach the best perinatal outcomes with the lowest rate of fetal loss and extreme prematurity [10-12]. The overall pregnancy loss rate before 24 weeks of gestation after SF was 4% in a series of 200 patients [10] and 7.5% in the largest collaborative experience of 402 women [11]. Also, Evans *et al.* showed a trend towards increasing loss rates with advancing gestational age at the time of the SF with a risk of fetal loss rate from 5.4% between nine to 12 weeks, to 9.1% when it is performed later than 25 weeks [11]. In the present case, the SF was performed at 13 weeks without any complications. Therefore, the present authors recommend that SF be performed as early as possible to minimize the risks in pregnancy and the psychological impact on women.

Although a short series with SF did not find a reduction in the rate of early preterm delivery [6], a recent systematic review with 58 dichorionic twin pregnancies discordant for anencephaly concluded that while SF does not reduce perinatal mortality, it does result in significantly longer gestations and higher birth weight than expectant management, as observed when compared to the two present cases [9].

In conclusion, despite the fact that this series is short, it seems that there are more advantages with SF than with expectant management in dichorionic twin pregnancies discordant for anencephaly. Nonetheless, the mother has the ultimate decision to take risks and her personal decision should always be respected.

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Severe headaches from intracranial hypertension (pseudotumor cerebri) abrogated by treatment with dextroamphetamine sulfate

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Summary

Purpose: To determine if sympathomimetic amines may relieve migraine headache pain from pseudotumor cerebri (PTC) similar to its effect on helping other types of migraine headaches that were recalcitrant to other therapies. *Materials and Methods:* A woman with severe migraine headaches which did not respond to treatment with acetazolamide was treated with dextroamphetamine sulfate sustained release capsules 25 mg daily. *Results:* The patient demonstrated marked improvement within a month. The marked decrease in headache pain has persisted over a year. Her papilledema also completely disappeared. *Conclusions:* The sympathetic neural hyperalgesia edema syndrome can manifest as PTC. Besides headaches, other symptoms that the patient manifested were part of this syndrome including chronic fatigue, inability to lose weight despite dieting, and backache. All of these additional symptoms also improved with sympathomimetic amine therapy.

Key words: Intracranial hypertension; Pseudotumor cerebri; Migraine headaches; Sympathomimetic amines.

Introduction

The gynecologist is frequently presented with a woman complaining of headaches. Physicians have a greater role than being merely "triage officers" and merely referring the woman to the "appropriate" specialist such as to a neurologist for headache complaints.

The gynecologist could consider that the headaches could be related to the estrogen in a birth control pill and may consider choosing an oral contraceptive with less estrogen, or a progesterone only pill, or trying an alternative form of contraception. Sometimes headaches may be part of the pre-menstrual syndrome and may improve with progesterone supplementation in the luteal phase.

Another common cause of headaches in women is related to hypofunction of the sympathetic nervous system [1-3]. This condition known as the sympathetic neural hyperalgesia edema syndrome is more familiar to gynecologists than neurologists or specialists in internal medicine because it is the most common remediable cause of pelvic pain [4].

Both the headache and pelvic pain usually improve considerably if not completely following treatment with the sympathomimetic amine dextroamphetamine sulfate [1-8].

By the gynecologist being familiar with this sympathetic nervous system disorder, the physician could institute sympathomimetic amines therapy without subjecting the woman to non-essential painful, expensive, and sometimes risky tests and still not come up with a solution. One may suggest, what if some serious pathological condition is missed by this approach, e.g. a brain tumor? The answer is that a serious pathologic state e.g. a brain tumor should not respond to dextroamphetamine sulfate.

The case below describes a woman whose headaches were related to the condition known as pseudotumor cerebri (PTC) (benign idiopathic intracranial hypertension - IIH). Though the testing allowed the diagnosis, unfortunately standard therapy failed to provide relief. However, sympathomimetic amine therapy completely corrected the problem. It not only corrected the headaches but the increase in cranial blood pressure and the papilledema also resolved.

Case Report

A 34-year-old woman with a history of a simple hysterectomy began experiencing sudden headaches which would have a sudden onset, were relatively severe in pain intensity, and would last several days at a time. The pain free interval in the beginning was about two weeks. After a year, the headaches were constant with no pain free intervals and were associated with chronic fatigue and inability to lose weight, despite dieting. The headaches were so severe at times, trips to the emergency room were needed for narcotics.

Though she was diagnosed with hypothyroidism, thyroid hormone replacement which allowed restoration of normal thyroid values did not help the headaches, backaches, chronic fatigue or weight problems.

She was referred to a neurologist and a neuro-opthalmologist. Significant papilledema was found. Spinal tap found an elevated

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pressure but no other abnormalities. A magnetic resonance imaging study of the brain failed to detect a tumor. She was thus diagnosed with PTC (benign IIH).

The woman at this point judged her headaches intensity on the basis of 1 to 10 as 10. Following combined therapy of topiramate and acetazolamide, she stated she had some relief and now judged the intensity as a 7.

She initially consulted our practice merely to manage her hypothyroidism having moved to a new state. She was advised that the thyroid dosage was correct. However she was advised that there was a good likelihood that treatment with dextroamphetamine sulfate may improve the chronic fatigue, backache, and inability to lose weight [8-10]. Furthermore, she was advised that the treatment may help the headaches in view of previous experience even though it had never been tried on headaches from PTC [1-3].

When she returned after one month of taking dextroamphetamine sulfate extended release capsules, she had marked improvement in the chronic fatigue and the headaches. The headaches had improved and were rated as a 5 on severity. The dosage was increased to 25 mg and when she returned the next month, the headaches had completely disappeared. When she had another fundoscopic evaluation by her neuro-opthalmologist, there was no longer any papilledema.

There have been no headaches now for over one year of treatment. Furthermore, she has lost 47 pounds. The headaches disappeared long before any significant weight was lost.

Discussion

IIH is a disorder characterized by increased intracranial pressure (ICP) of unknown cause, predominantly seen in women of childbearing age and associated with a history of recent weight gain [11]. The concept of raised ICP in the absence of a space occupying lesion was first introduced by Nonne [12] as "pseudotumor cerebri".

The term "benign intracranial hypertension" became popular and was often used interchangeably with PTC. The condition was considered "benign" in comparison with cases of tumor [13], but it has been argued that loss of visual function in up to 25% of cases and progression to blindness if untreated means that it should not be considered "benign" as far as visual function is concerned.

Recently publications have looked again at the question of weight gain in IIH, some suggesting pathogenic mechanisms which may explain how weight gain can lead to raised ICP. Others suggested the reverse-that raised ICP is the cause of weight gain. However there is no convincing evidence that weight loss results in lowering of the ICP according to Fraser and Plant [14].

Based on this case, since the headaches improved, before there was significant weight loss the response in this case suggests that sympathetic nervous system hypofunction can be the etiology for benign IIH and weight gain is merely a frequent associated issue with this syndrome called sympathetic neural hyperalgesa-edema syndrome related to the edema. It is not clear if the improvement in headaches is related to inhibition of absorption of toxins into brain tissues by correcting cellular permeability issues or by lowering ICP or both [15].

Acetazolamide (a carbonic anhydrase inhibitor) has been considered by the neurologist as the mainstay of medical therapy. Topiramate has also been used. Two drugs not tried on this woman are frusemide and bendroflumerthiazide. Some think the advantage of topirmate over acetzolamide is that the former can cause weight loss and losing weight my also help this condition [16]. However, the subject of this case report did not lose weight until she was treated with dextroamphetamine sulfate.

There are surgical treatments also including lumbo-peritoneal shunt, ventriculo-peritoneal shunt, and optic nerve sheath fenestration [17]. Other surgical options considered have been bariatric surgery [18]. Also in some cases where venous sinus stenosis is diagnosed, endovascular stenting has had some success [17].

Because of potential blindness the word benign should probably be removed from the name of IIH. The potential seriousness of this condition and the probabilities of missing a brain tumor could be used to justify the need to refer to a neurologist. Even IIH seems at first glance too complicated for the gynecologist.

However again, to re-iterate it is unlikely that headaches from a brain tumor would respond to dextroamphetamine sulfate. It is not clear if rare causes of IIH e.g.: giant arachnoid granulation in the left dominant transverse sinus or other causes of IIH e.g.: dural venous sinus stenosis would even respond to sympathomimetic amine therapy [11].

On the other hand, when the woman returned to her neuro-opthalmologist, she did not seem interested in the patient's story of marked improvement with dextroamphetamine sulfate. Instead she spend only five minutes, did a fundoscopic exam, told the patient her papilledema was gone, and to return in six months for a repeat fundoscopic examination. The physician just assumed that her prescribed therapy finally worked.

Similarly an unreported case of a woman with 20 years of serve migraine headaches that were refractory to standard therapy was told by her neurologist that he believed that she will eventually develop multiple sclerosis and that was the cause of her headaches. Twenty years later she never developed multiple sclerosis. She heard that dextroamphetamine sulfate therapy could help headaches and she was placed on it. Within two weeks, her headaches of 20 year duration, cleared completely. When she advised her neurologist of these events, he advised her to stop the drug immediately because there are no prospective evidenced based randomized controlled studies proving its efficacy. She actually listened to him, stopped the dextoamphetamine sulfate, and within a week her headaches returned. After two months of severe headaches she returned to our practice, was placed on the amphetamine, and was immediately relieved again of her headaches.

Thus until this condition related to sympathetic nervous system hypofunction becomes known by neurologists and internal medicine specialists, it is important for the primary

care physician of women, i.e., the gynecologists, to either initiate therapy with sympathomimetic amines first without consulting a neurologist or referring the women to a neurologist, but advise the woman before proceeding with any suggested tests or therapies, to return to the gynecologist to decide if the proposed therapy seems reasonable, or should sympathomimetic amines be tried first?

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Successful rescue hysteroscopic resection of a cervical ectopic pregnancy previously treated with methotrexate with no combined safety precautions

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Summary

Background: Cervical pregnancy (CP) is a life-threatening condition that represents less than one percent of all ectopic pregnancies. Transvaginal sonography (TVS) is the gold standard for an accurate diagnosis. For hemodinamically stable women the available treatments involve a medical therapy, alone or in combination with interventional measures (hysteroscopy, angiographic embolization or laparoscopic ligation of uterine arteries). *Materials and Methods:* The authors describe a CP unsuccessfully treated with methotrexate (MTX), but resolved with hysteroscopy. *Case Report:* A nulliparous woman arrived with low abdominal pain without vaginal bleeding at six weeks of amenorrhea. TVS revealed a gestational sac implanted in the isthmic cervical region, with a serum β-hCG of 1,100 mUI/ml, that raised to 4,274 mUI/ml in a week, despite one intrasaccular-MTX injections and two systemic doses. The authors arranged for a hysteroscopic resection with no previous dilatation of the cervix. They did not adopt any safety precautions to their procedure. *Conclusion:* It is difficult to define the exact role of hysteroscopy regarding CP. Despite some authors dispute on its complementary function to MTX, the authors believe that it could be used as a rescue method in case of MTX failure. The final aims of a proper management are to minimize the risk of haemorrhage and preserve women's fertility.

Key words: Cervical ectopic pregnancy; Methotrexate; Hysteroscopy; Fertility.

Introduction

Ectopic cervical pregnancy (CP) is a rare life-threatening condition with the incidence varying from 1:1,000 to 1:18,000 reported pregnancies [1,2], and represents almost less than one percent of all ectopic pregnancies [3]. There is a growing frequency of CP that could be due to an improved incidence in patients who undergo assisted reproductive technology procedures for infertility treatment. Other risk factors are the use of intrauterine device, previous abortion, uterine curettage, and previous cesarean section. In the past, an emergency hysterectomy was often the only available choice because of profuse hemorrhage that accompanied the attempts of removal of a suspected incomplete abortion. Advances in ultrasonography (US) technology and the availability of quantitative beta-human chorionic gonadotropin (β-hCG) have made diagnosis of CP possible at an early gestational age. According to a recent review, a CP could be diagnosed on sonography if the following criteria are fulfilled: 1) an empty uterus, 2) a barrel-shaped cervix, 3) a gestational sac present below the level of uterine arteries, 4) absence of the sliding sign (when pressure is applied to the cervix using the probe, the gestational sac slides against the endocervical canal in a miscarriage, but does not in an implanted cervical pregnancy) and 5) blood flow around the gestational sac on color Doppler [4]. Available treatments usually consist of a combination of medical measures, such as methotrexate (MTX), misoprostol, mifepristone, and interventional measures, for example the US-guided injection of MTX or potassium chloride (KCl) directly in the gestational sac, curettage and tamponade, and needle aspiration of the products [5, 6]. Other authors have reported the use of laparoscopic surgery, hysteroscopic excision, and angiographic uterine artery embolization, with a high degree of success and minimal morbidity [3, 7-10].

In the present report the authors describe a case of a CP unsuccessfully treated with MTX (both intrasaccular and systemic), but resolved with a hysteroscopic resection of the gestational sac.

Case Report

A 40-year-old pregnant nulliparous woman with a history of salpingectomy for pelvic inflammatory disease, who conceived following in vitro fertilization-embryo transfer (IVF-ET) and freezing blastocyst transfer, was referred to the present center with a recent history of low abdominal pain without vaginal bleeding. The gestational age, according to her last menstrual period, was six weeks. Transvaginal sonography (TVS) revealed the possible diagnosis of a CP on the basis of the presence of a gestational sac located in the cervical canal, below the internal os, and an empty uterine cavity; there was no embryonic heart rate (EHR) and the initial serum β-hCG was 1,100 mUI/ml. The patient was hemodinamically stable for two days, with an increased serum β-hCG (2,023 mUI/ml) and was counselled about possible options and

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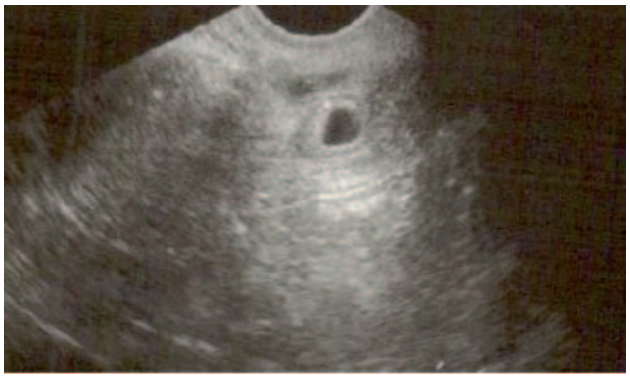

Figure 1. — Transvaginal ultrasound showing the gestational sac implanted in the cervical-isthmic region.

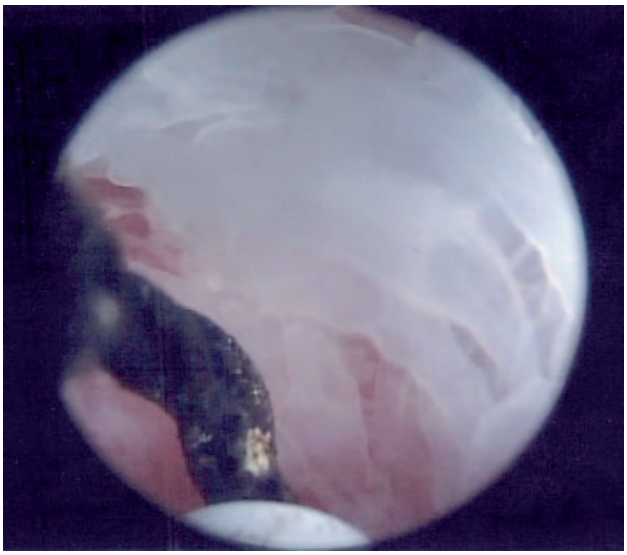

Figure 2. — The cervical canal vision from the five-mm Bettocchi hysteroscope, right before the resection of the cervical pregnancy.

complications for this pregnancy. The third day a new TVS revealed a six-mm gestational sac implanted in the isthmic cervical region, with yolk sac and still no cardiac activity (Figure 1). After these evidences the authors decided to perform a US-guided intrasaccular MTX injection (50 mg on two ml of NaCl), explaining all the possible complications. During the first postoperative day the patient was stable, but her serum β-hCG increased (2,742 mUI/ml) and a new TVS showed a seven-mm gestational sac with a three-mm yolk sac, with a fetal pole of 1.5 mm, and a discover of EHR. On the third day post-intrasaccular MTX injection, another TVS revealed an hematoma in the inferior pole (probably related to the operative procedure). Moreover, ββ-hCG increased to 3,979 mUI/ml, so they decided to repeat MTX, but with a systemic dose (1.5 mg/kg), since another intrasaccular injection was impossible to perform due to the cervical hematoma. Three days after they decided to perform a second systemic MTX dose (the third, counting the intrasaccular injection) because serum ββ-hCG had increased (4,274 mUI/ml), EHR was still present and the patient became emotionally unstable. The following day serum ββ-hCG slightly decreased (3,979 mUI/ml), but an additional TVS still displayed a 18-mm gestational sac with persistent cardiac activity, so the authors arranged for a hysteroscopic resection of the CP. Under general anesthesia, they inserted into the cervical canal a five-mm Bettocchi hysteroscope, with no previous dilatation of the cervix, using the vaginoscopic approach; they introduced through the operative channel a 5F bipolar electrode, and resected the implantation site under direct visualization of the chorial villi (Figure 2). A second look showed an empty cervical canal. The day after serum ββ-hCG dropped down to 1,566 mUI/ml. The postoperative course was almost uneventful, so the patient was dismissed with a planned follow-up based on TVS examination and ββ-hCG measurements. After two months ββ-hCG was 10 mUI/ml and a TVS exam revealed a small non-vascularized lesion, that was easily removed by an office hysteroscopy. ββ-hCG measurement is now actually negative and the patient is trying to become pregnant.

Discussion

This case report shows that an early CP can be treated with a conservative hysteroscopic resection of the gestational sac after the failure of MTX administrations (local or systemic).

Several studies have reported that MTX therapy has a 91% success rate, but also carries the potential risk of MTX-related disadvantages: the need of adjuvant surgical or endoscopic procedures (34%), a mean longer time for ββ-hCG negativization, a long time to return of menstruation, and a possible leukocytopenia [11]. Moreover, the efficacy of MTX could be related to some conditions that have to be respected: in fact a serum ββ-hCG >10,000 mUI/ml, a gestational age > nine weeks, the presence of EHR or crown-rump length (CRL) > ten mm have been shown to be accompanied by a higher rate of MTX failure and may need additional interventions [12]. MTX can be administrated by two different routes. Although the systemic administration is easier to perform than the local intra-amniotic injection [13], it is less effective, it needs an increased dosage if compared with the intrasaccular procedure, it has more side-effects, and also takes more days for aberrant trophoblast to be eradicated through MTX-induced antimetabolite effects [12].

If MTX can be considered the most conservative approach regarding CP, on the other hand it can be taken into account as conservative procedures all minimally invasive techniques that could preserve women's fertility, such as hysteroscopy, discussed for the first time in 1992 [14]. Since the risk for hemorrhage in a CP is high, all approaches need to be cautious. In fact some authors dispute on the safety of the endoscopic route alone, so several reliable precautions have been described, such as laparoscopic uterine artery ligation [7], or transfemoral uterine artery embolization [9, 10]. It is not clear if all these described safety precautions are necessary and it resulted that the endoscopic route is safer. In fact, Jozwiak *et al.* did not report any complications describing a heterotopic CP treated only with hysteroscopy, without previous dilatation or security

adjustments [15]. In addition, all these safe maneuvers may result in uterine hypoperfusion and possible subsequent hypofertility [16].

The present authors' clinical management initially resorted to conservative MTX therapy, because CP respected the inclusion criteria for a successful pharmacological treatment. Considering that ββ-hCG increased and that EHR was still persistent (despite three MTX doses), they changed their strategy and opted for the hysteroscopic approach. This procedure resulted to be secure and with low operative time. In fact, a previous dilatation could provoke a severe hemorrhage, while the cervical distension is not triggered using a five-mm hysteroscope. For these reasons the authors did not adopt any safety precautions with their hysteroscopy; in their opinion the laparoscopic ligation of uterine arteries is too invasive and takes a long mean operative time, while angiographic embolization requires radiologic facilities with expertise and specialized instrumentation, not available in every center. Moreover it requires to be confirmed whether hysteroscopy could become a secure one-step procedure in selected cases without previous dilatation, surgical or medical precautions.

The final aims of a proper management are to concentrate on minimizing the risk of hemorrhage, eliminating the gestational cervical product, and preserving women's fertility. So an early diagnosis of a CP influences the choice of treatment, that has to be customized based on the women's condition, their clinical manifestations, the availability of different procedures and, finally, the clinician's experience. Considering its small incidence, only prospective randomized multicentric studies will be able to determine its proper management. In conclusion, with this case report the authors would like to suggest a rescue role for hysteroscopy after a MTX failure. This approach could be a safe, simple, rapid, and resolving treatment regarding uncomplicated cervical ectopic pregnancy.

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Prenatal diagnosis of multiple fetal anomalies in naphthalene-addicted pregnant women: a case report

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Summary

Background: Naphthalene is one of the abused inhalants. It has been associated with acute and chronic health problems. To the authors' knowledge, prenatal exposure to naphthalene has never been discussed in humans. *Case:* The authors discuss a case of naphthalene-addicted pregnant women with multiple fetal anomalies. At 15 weeks gestation, ultrasound screening demostrated multiple fetal anomalies: anencephaly, scoliosis, diffuse subcutaneous edema, flexion contracture of lower extremities, and hypoplastic left ventricle. Four weeks later obstetrical ultrasonography revealed that there was no fetal cardiac activity. The patient had a medical abortion. *Conclusion:* A stronger knowledge basis regarding naphthalene-related fetal anomaly is required to ensure accurate direct link, however the probability of naphthalene-related fetal anomaly must be considered.

Key words: Naphthalene abuse; Multiple fetal anomalies; Pregnancy.

Introduction

Inhalant abuse is a significant problem, especially in adolescents [1]. The most commonly abused inhalants are chemical products that are available, accessible, inexpensive, and legally obtained. Naphthalene, one of the abused inhalants, is an aromatic hydrocarbon and it is metabolized to naphthol and naphthoquinone by the liver and these metabolites are excreted in the urine [2,3]. Hepatic injury and hemolytic anemia are the most common toxicities. Renal insufficiency, cataract formation, methemoglobinemia, aplastic anemia, and cardiac dysrhythmias are other less frequent related toxicities related to naphthalene abuse. Central nerve system toxicities, such as slurred speech, ataxia, and coma can also occur. Peripheral neuropathy has also been reported [4].

In Turkey, naphthalene is a popular fumigant insecticide commonly used to protect wool garments from bite damage. It is also the major component of glue, spray paints, nail polish remover, room fresheners, and gasoline.

To the authors' knowledge, prenatal exposure to naphthalene has never been discussed in humans. They present a case of a naphthalene-addicted pregnant women with multiple fetal anomalies.

Case Report

A 19-year-old, gravida 1 woman was admitted to the present clinic for antenatal care. Gestational age was seven weeks and two days according to last menstrual period. Ultrasound revealed fetal cardiac activity and crown rump length (CRL) of the fetus measured as seven weeks and four days. There was no pathologic sign in the physical examination of the pregnant woman. Her husband declared that she had a history of substance abuse including naphthalene by inhalation and had had rehabilitation for this problem. She stated that she had not been using naphthalene for two months. The authors recommended the patient psychiatric support during the pregnancy period, but refused to see a specialist and also treatment. The antenatal laboratory data showed that complete blood count, blood electrolytes, and hepatic and renal function tests were normal. Antenatal care visits were also planned but the patient was not fully cooperative. Eight weeks later -at 15 weeks- she was admitted to the present clinic with nausea and vomiting. Physical examination was normal, but the authors recorded that she had lost eight kg in that time period. She refused to answer the questions regarding naphthalene inhalation. The authors re-evaluated the laboratory tests including, complete blood count, blood electrolyte, hepatic, renal, and thyroid function tests. All of them were normal.

A detailed ultrasonographic evaluation of the fetus was performed and multiple fetal anomalies were detected. Anencephaly (Figure 1) and scoliosis in vertebral spine were diagnosed. Diffuse subcutaneous edema was also present (Figure 2). Lower extremities of the fetus were in flexion contracture. Fetal echocardiography demonstrated hypoplastic left ventricle and pleural effusion.

The patient and family were informed regarding lethal fetal anomalies and counseled regarding the option of termination and again they recommended psychiatric support. They did not, however, accept both.

Four weeks later obstetrical ultrasonography revealed that there was no fetal cardiac activity. The patient had a medical abortion in a nearby county hospital.

After termination of pregnancy, the prenatal diagnosis of multiple anomalies were confirmed by autopsy, with the identification of prenatal sonographic findings.

Discussion

The clinical literature regarding prenatal organic solvent exposure is limited. The most popular abused inhalants is toluene [5] and most of the studies assessing

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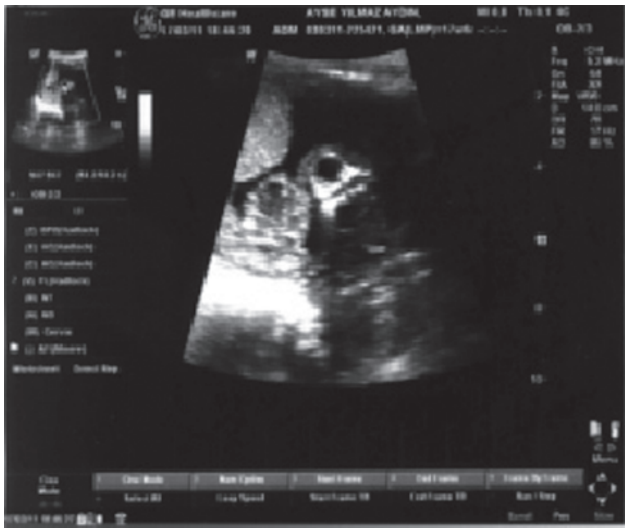

Figure 1. — Anencephaly is illustrated.

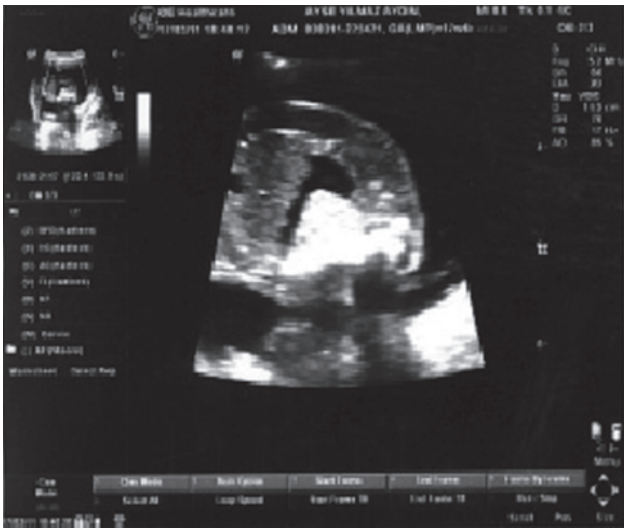

Figure 2. — Diffuse subcutaneous edema is shown.

perinatal organic solvent exposure are about toluene. There are some cases also reporting toluene-related embryopathy [6-8]. Perinatal death related to very high levels of maternal solvent exposure, typical of abuse, has also been reported and there are reports that surviving neonates show evidence of morphological teratogenicity. Prematurity or growth retardation, microcephaly with severe facial dysmorphology (deep-set eyes, small face, low-set ears, micrognathia), and spatulate fingertips and small fingernails are the anomalies that had been reported in affected infants [9]. Follow-up evaluations of the toluene-exposed children up to three years of age revealed developmental delays, language impairment, hyperactivity, cerebellar dysfunction, and postnatal growth retardation.

Naphthalene has rarely been abused. Most of the reports of toxicity are acute and accidental. To the authors' knowledge, this is the first case regarding perinatal toxicity. Because of several confounding factors, it is difficult to establish a direct link between abuse of naphthalene and fetal anomaly. Many inhalant abusers can use various products since they have problems with accessibility. Concomitant abuse of other drugs and alcohol is common and can also be another confounding factor. Changes in solvent formulations, impurity within solvents, and most importantly, genetic predisposition and preexisting medical conditions, are also confusing. There is no study that evaluates high-dose prenatal exposure to naphthalene in animal models. The presented patient has no other risk factor for fetal anomaly, such as diabetes mellitus, advanced maternal age, infection, alcohol abuse, and family history.

In summary, a stronger knowledge base regarding naphthalene-related fetal anomaly is needed to ensure accurate direct link, but the probability of naphthalene-related fetal anomaly must be considered.

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Can laparoscopic removal of Essure device before embryo transfer correct poor reproductive outcome pattern in IVF? A case report

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Summary

Objective: This report describes a successful surgical approach to multiple in vitro fertilization (IVF) failures in the setting of hydrosalpinges, which had been previously treated with Essure inserts. *Materials and Methods:* A non-smoking 33-year-old Caucasian G2 P0020 (body mass index: BMI = 22) attended for second opinion. Her history was significant for bilateral hydrosalpinges having been noted on hysterosalpingogram two years earlier. This was managed by hysteroscopic placement of Essure inserts bilaterally. One year later, and now with Essure in situ, the patient completed three IVF cycles elsewhere. Her first and third IVF attempts resulted in biochemical pregnancy, while human chorionic gonadotropin (hCG) was negative after the second cycle. Upon presentation at the authors' center and before beginning a fourth IVF cycle, further testing and surgical removal of the Essure devices was recommended. *Results:* Repeat hysteroscopy was unremarkable; laparoscopic bilateral salpingectomy and extirpation of Essure implants was accomplished without difficulty. Following menses, the patient initiated IVF with three embryos transferred. At day 60, a single intrauterine pregnancy was identified with positive cardiac activity (rate >100/min). Her obstetrical course was uneventful; a healthy 4,195 gram male infant was delivered (breech) by Cesarean at 40 weeks' gestation. *Conclusion:* Essure inserts comprise inner fibers of polyethylene terephthalate, a stainless steel coil, and a nickel-titanium coil. The product received FDA approval as a contraceptive in 2002 although its use for hydrosalpinx remains off-label. While successful outcomes with IVF following Essure placement have been reported, this is the first description of pregnancy and delivery from IVF after Essure removal. Essure may be considered for sterilization when laparoscopy is contraindicated, but experience with its use specifically for treating hydrosalpinges before IVF is limited. This observed association between prior poor IVF outcomes and Essure with subsequent delivery after surgical Essure removal is the first of its kind to be reported, and warrants further investigation.

Key words: Essure; Hydrosalpynx; IVF; Recurrent miscarriage; Laparoscopy.

Introduction

Hydrosalpynx identified during the evaluation prior to in vitro fertilization (IVF) represents a significant finding [1]. Most research to date has recommended surgical correction of such tubal pathology before embryo transfer [1,2]. There is now general consensus that surgical treatment should be considered for all women with hydrosalpinges before IVF treatment. Room for debate does exist, however, on how best to carry out such surgery. A recent comprehensive review on this topic [3] concluded that laparoscopic tubal occlusion or laparoscopic salpingectomy appropriate interventions are to improve IVF pregnancy rates in women with hydrosalpinges. It should be noted that simple proximal tubal occlusion has been recognized as an effective alternative to salpingectomy, if the latter were technically difficult or impossible to complete for other reasons [4]. Because proximal tubal occlusion can also be achieved by the non-incisional hysteroscopic insertion of metallic microinserts (Essure), this non-laparoscopic approach has attracted considerable attention. Early papers describing abdominal operations for correction of hydrosalpynx before IVF were all predicated on the understanding that pre-IVF patients are willing and able to undergo laparoscopy, yet this may not always be the case. Indeed, hysteroscopic management brings several advantages over laparoscopy by eliminating the need for abdominal access [5,6], reducing overall cost [7-9], and minimizing anesthesia requirements [10]. These characteristics have enabled a small but growing experience with Essure placement specifically for proximal tubal occlusion for women with hydrosalpynx before IVF. Thus far, all such publications have been favorable [11-17]. In this report, we describe IVF preceded by the surgical removal of Essure implants where multiple poor outcomes had occurred with Essure *in situ*. After three failed IVF attempts, our patient conceived on her fourth IVF cycle and delivered once the Essure devices were excised.

Case Report

A healthy, non-smoking 33 year-old Caucasian G2 P0020 with regular menses attended for reproductive endocrinology consultation and second opinion. Physical examination was unremarkable and BMI was 22kg/m^2. Past medical history was significant

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for bilateral hydrosalpinges which were noted on hysterosalpingogram performed two years before initial consult with the present authors. The patient subsequently underwent a laparotomy for bilateral ovarian cysts, but the fallopian tubes were apparently not accessible. The patient had been advised that communicating hydrosalpinges required treatment before IVF, and was offered an office hysteroscopy procedure (Essure) as a non-laparoscopic way to resolve the tubal fluid.

The patient underwent bilateral Essure placement and tolerated the procedure well. The patient then waited one year and had a repeat hysterosalpingogram, which confirmed bilateral proximal tubal occlusion secondary to the device placement. Next, the patient embarked three fresh IVF cycles. All three of these attempts were completed at the same institution with similar monitoring practices and identical ovulation induction regimes. The patient's response to controlled ovarian hyperstimulation was adequate and there were no complications. Evaluation of the male partner at the present center agreed with the previous assessments and confirmed normal semen parameters.

The first and third IVF attempts resulted in biochemical pregnancy (not requiring D&C), although serum hCG was zero following the second IVF cycle. Luteal phase support remained unchanged for the three IVF cycles. There were no frozen surplus embryos available from any of these IVF treatments.

Upon presentation at our unit and before beginning a fourth (fresh) IVF cycle, further testing and surgical removal of the implants was discussed. An autoimmune panel and thrombophilia testing did not reveal any abnormality, and karyotypes on both partners were also normal. Repeat hysteroscopy was unremarkable; laparoscopic bilateral salpingectomy and extirpation of Essure implants was accomplished without difficulty (Figure 1). Following menses, the patient initiated IVF with three embryos transferred. Transvaginal ultrasound performed on day 60 revealed a single intrauterine pregnancy with positive cardiac activity (144/min). The patient's obstetrical course was uneventful and a healthy male infant (birth weight 4,195 g) was delivered by Cesarean for breech presentation at 40 weeks' gestation. The placenta was delivered without difficulty. Mother and baby continue to do well.

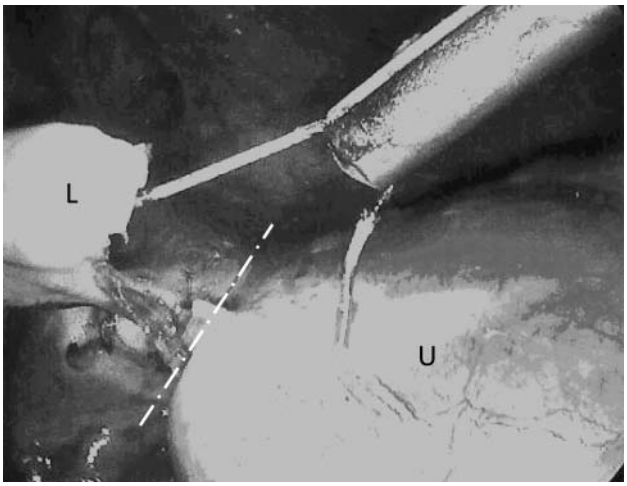

Figure 1. — Left Fallopian tube (L) divided near the uterotubal junction (dashed line), with removal of the Essure device using a five-mm laparoscopic grasper. The same approach was used for the contralateral tube. Interior and exterior contours of the uterus (U) were normal.

Discussion

The presence of fluid-filled tubes is a reliable marker of pelvic pathology, as hydrosalpinx fluid is now recognized as antagonistic to embryo implantation [18, 19]. Subsequent research has found IVF pregnancy rates in the presence of hydrosalpynx fluid reduced by up to 50% compared to age-matched women without this finding [20]. This adverse effect of communicating hydrosalpinges on embryo survival and/or implantation rates in IVF has been reported by numerous investigators [21-24], supporting the recommendation to take surgical action before IVF. For most IVF patients, salpingectomy or proximal tubal occlusion by laparoscopy is the operation usually recommended to address the hydrosalpynx problem [2,25]. However for patients who are poor candidates for laparoscopy, a non-abdominal approach to achieve tubal occlusion before IVF was needed.

The arrival of the Essure device, a non-incisional procedure for tubal sterilization which is performed hysteroscopically in an office setting, drew interest as one way to fill this niche [5]. Clinical investigation of Essure began in 1996, and it received FDA approval for use in permanent sterilization in 2002 [15]. Although the product was approved for a contraceptive indication rather than to treat hydrosalpynx before IVF, Essure has consistently provided satisfactory results in this off-label application [11-17].

One of the first studies to detail post-Essure intrauterine cavity status [13] used a second-look hysteroscopy design, with repeat hysteroscopy between four and 43 months after initial Essure placement. The investigators reported data on 22 patients, and complete tissue encapsulation of both micro-inserts had already occurred in 17% of cases when reexamined within 12 months or less. Among study patients reevaluated 13-43 months post-Essure insertion, complete encapsulation was noted in 25% [13]. Several investigators have offered support for exclusive use of Essure specifically to correct retrograde flux of hydrosalpynx fluid before IVF [11-17].

Despite promising early results with Essure placement before embryo transfer, the approach has not been systematically reviewed and remains off-label. Indeed, the 2010 Cochrane review [3] found that surgical treatment should be considered for all women with hydrosalpinges before undergoing IVF treatment, with an emphasis on laparoscopic tubal occlusion or laparoscopic salpingectomy to improve pregnancy rates with IVF for women with hydrosalpinges. With regard to postoperative intrauterine inflammatory mediators impacting endometrial receptivity and embryo implantation, more data are needed to establish what difference may exist after laparoscopic vs. hysteroscopic interventions.

Against this background, the present case is the first report to describe unsatisfactory IVF outcomes in association with proper placement of Essure inserts for management of hydrosalpynx. Because Essure is a permanent sterilization method, the product is not supposed to be removed and guidelines for surgical extirpation of the device are currently lacking. While it is encouraging that others have shown this off-label use of Essure can lead to pregnancy and delivery [11-17], for the present patient, delivery was possible only after laparoscopic removal of Essure and salpingectomy. Indeed, except for these surgical tubal manipulations, no other aspect of IVF treatment changed for the present patient when her first three cycles are compared to the fourth successful attempt. Because hysterosalpingogram and hysteroscopy were normal following Essure insertion, the authors' suspicion for improper placement of the device or reflux of tubal fluid was low. Nevertheless, the present case frames this key question: is it possible that even in the setting of good placement technique and complete device engraftment and encapsulation, can the Essure device exert some type of inflammatory endometrial contraceptive effect sufficient to antagonize embryo implantation? The current report offers a crucial counterpoint to the emerging literature on Essure, and suggests that laparoscopic removal of Essure microinserts before embryo transfer can correct a recurrent poor outcome pattern in IVF.

Conclusions

Given that the extant literature on using Essure for hydrosalpynx before IVF has been, until now, uniformly reassuring, the present patient's disappointing IVF results all occurring with Essure implants *in situ* were surprising. Fortunately laparoscopy was not contraindicated in this case, and surgical removal of both Essure implants could be achieved without complication. Had the present patient been unable or unwilling to undergo abdominal surgery, our therapeutic options would have been limited to surrogacy or to repeat her embryo transfer (a fourth time) with Essure implants still present—neither were attractive alternatives on this occasion. Based on the new findings presented here, we believe that IVF patient counseling should discuss the potential impact on IVF outcome when Essure is used for this off-label indication. Further clinical experience will be welcome as IVF outcomes following Essure use for this indication continue to be monitored.

Acknowledgements

SAS and ACP were consultants associated with the case, and RDS was principal surgeon. ESS conceived of the project and developed the manuscript. All authors read and approved the final submission.

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Premature ovarian failure in a 17-year-old woman

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Summary

Introduction: Premature ovarian failure (POF) in a healthy adolescent is a rare event. It is diagnosed by the presence of amenorrhea, hypoestrogenism, and elevated follicle-stimulating hormone (FSH) levels before the age of 40. *Case:* The patient presented with amenorrhoea at 17 years after identifying a change from her regular to irregular and metrorrhagic cycles. No positive medical history was noted regarding smoking, chemotherapy, radiation or autoimmune diseases and the physical examination was normal. Her family history revealed that both her maternal aunt and grandmother were affected by POF, but the karyotype test was normal and the FMR1 screening premutation test was negative. The patient underwent an ovarian biopsy which revealed the absence of functional follicles. She began a replacement therapy with estroprogestogens and she was informed about the most successful means to start a family, including adoption and oocyte donation. *Conclusion:* POF is a heterogeneous, multifactorial, and poorly understood condition that involves medical concerns, psychological sphere, and sexuality of the affected patients. Management should be directed at symptoms resolution, bone protection, and psychosocial support for women facing this unexpected and devastating diagnosis.

Key words: Premature ovarian failure; FMR1 test; Hormone replacement therapy; Infertility.

Introduction

Premature ovarian failure (POF) is a clinical condition characterized by the presence of primary or secondary amenorrhea for at least four months, hypoestrogenism, and elevated serum gonadotropin concentrations due to cessation of ovarian function before the age of 40.

The diagnosis is confirmed by serum follicle-stimulating (FSH) levels in a classical menopausal range (> 40 IU/l) in two measurements at least one month apart [1].

The condition differs from menopause because of varying and unpredictable ovarian function in approximately 50% of cases, and about five to 10% of women conceive and deliver a child after they have received the diagnosis [2].

POF incidence in patients with 46, XX karyotype was estimated in around 1:1,000 women under 30 years old, 1:250 around 35 years old, and 1:100 at 40 years old [3].

Multiple causes of POF can be defined and result in follicle depletion and/or defects in the follicular development. POF may occur due to chromosomal, genetic, autoimmune, metabolic (galactosaemia), infectious (mumps), and iatrogenic (anticancer treatments) causes, but a large proportion of cases remains idiopathic.

Although most cases of primary ovarian insufficiency occur sporadically, there is a positive family history, with an affected first-degree relative, in approximately 10 to 15% of cases [4]. The standard diagnostic procedure in the case of young women with primary or secondary amenorrhea should include a cytogenetic examination and tests for the FMR1 premutation, especially in the case of women under the age of 25.

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Case Report

A 22-year-old caucasian woman had experienced secondary amenorrhea from the age of 17.

She had menarche at the age of 13 and reported regular menses for the following two years. Later she presented metrorrhagic cycles until cessation of ovarian function at the age of 17, when she began experiencing hot flashes and loss of libido. She had received an estro-progestogens association therapy (ethinyl estradiol 0.020 mg and drospirenone three mg for six months, followed by medroxyprogesterone acetate and ethinyl estradiol for six months) which resulted in cyclical bleeding, but she remained anovulatory.

Her careful family history indicated that her maternal aunt and grandmother went through menopause before the age of 40. She was nulliparous. No positive medical history was noted regarding smoking, chemotherapy, radiation or autoimmune diseases.

Her physical examination revealed a healthy appearing woman with body mass index (BMI) of 20 kg/m^2, normal genitalia, and Tanner stage V development. Pelvic ultrasonography showed the body of the uterus to be normal in profile and dimensions, with a homogeneous thin endometrium. Both ovaries were reduced in volume (right ovary 2.1 cm^3, left ovary2.4 cm^3) with the absence of growing antral follicles.

Hormonal pattern cycle was: FSH 60: UI/l, luteinizing hormone: 42 UI/l, estradiol: 42 pg/ml, progesterone: 1.7 ng/ml, anti-Müllerian hormone: 0.4 ng/ml. Serum levels of thyroid stimulating hormone, fT3, fT4, prolactin, androstenedione, free testosterone, and total testosterone were within normal range.

No anti-thyroid, anti-antiadrenal, and anti-ovarian antibodies were found in the blood. Rheumatoid factor (RF) was negative. After a thrombophilic screening the patient showed hyperhomocysteinemia and an homozygous mutation in methylenetetrahydrofolate reductase (MTHFR).

The patient performed a karyotype test on peripheral blood resulting in a normal chromosomal pattern. The fragile X mental retardation 1 (FMR1) screening premutation test was negative.

She underwent a laparoscopy with an ovarian biopsy. The uterus appeared normal for morphology and volume with normal fallopian tubes and streak ovaries. The ovarian specimens were

routinely processed in paraffin and were stained by hematoxylin and eosin (H&E) staining (Figure 1).

At the histological examination, in the right ovary it was observed an atrophic cortex without follicular formations and corpus albicans. In the left ovary it was possible to distinguish an atrophic cortex with residual corpus albicans. On the histological section, non-functional primordial follicles with a single layer of granulose cells were identified. At the immunohistochemical analysis they were c-kit and PLAP negative.

The patient was discharged to home on the second post-operative day and she began a replacement therapy with estro-progestogens associated with folic acid and iron supplement.

A psychologist's consultation was performed to offer an emotional support and to discuss the implications of oocyte donation and adoption.

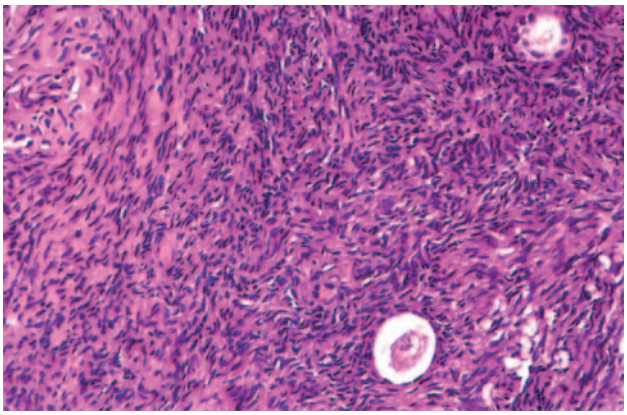

Figure 1. — Histopathology of the left ovary (H&E staining): atrophic cortex with non-functional primordial follicles.

Discussion

About one percent of women in the general population experiences cessation of ovarian function under the age of 40. Many women who presented a spontaneous POF, in a percentage from four to 31 percent, had an inherited character [5, 6].

The patient of this case report showed a strong association between her idiopathic form of POF and its manifestation in her family, as demonstrated by the fact that her maternal aunt and grandmother went through menopause before 40 years. There is a clear correlation between menopausal age of mother and daughter probably due to genetic mutations or deletions. The physiological decline of quality and quantity of oocytes causes a progressive loss of female fertility depending on the age of the patient [7]. The lack of specific markers of ovarian failure reveals the difficulty to anticipate how fast a patient with elevated FSH serum levels will develop an ovarian insufficiency [6]. Studies of pedigrees on affected families show a mode of inheritance suggestive of autosomal dominant sex-limited tranmission or X-linked inheritance with incomplete penetrance [8]. In recent years, the candidate gene approach has aided to identify genes and pathways involved in POF. Therefore, the pathogenic mechanism still remains unknown in most of the cases. However, when a genetic alteration is found in a woman, family counselling can be useful to predict the female relatives who are at higher risk for POF and fertility loss in young age. The FMR1 premutation accounts for 1.0% - 7.5% of sporadic and 13% of familial cases of POF. Thus, according to the American College of Obstetrics and Gynaecology, testing for the fragile X premutation should be recommended in all women with POF [9]. In addition, the American College of Medical Genetics recommends that testing should be considered in 'women who are experiencing reproductive or fertility problems associated with elevated FSH levels, especially if they have (a) a family history of premature ovarian failure (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation' [10].

Women with POF should ideally be managed within specialist multidisciplinary teams to address their complex physical and psychological needs. Therapeutic goals of POF are emotional health, hormone replacement therapy (HRT), maintenance of bone health, and concern about associated disorders, such as cardiovascular and metabolic diseases.

Many women with POF would benefit from symptom relief by the use of exogenous steroids, to compensate for the loss of ovarian estrogens, and possibly progesterone and androgens. Menopausal symptoms, such as hot flushes, night sweats, fatigue, sexual disfunction, and vaginal dryness, can be alleviated by estrogen replacement, such as sequential HRT or oral contraceptive pill. Women who are concerned about avoiding pregnancy are often advised to take the combined oral contraceptive pill. For women with an intact uterus, estrogen should be administered in combination with a progestin to avoid endometrial hyperplasia [11]. The dosage and the route of administration of HRT is extremely complex because of the chronicity (many years of treatment) and because during that period of time many changes take place at both the physical and psychological level. An HRT regimen should be based on the individual preferences of each patient who should be encouraged to undertake a trial and error approach through the wide variety of products available. There is no doubt that all women with POF must replace their missing steroid hormones until the age of natural menopause [12].

To date, no data are available to evaluate the impact of HRT on risks encountered by postmenopausal women, including the development of breast cancer, endometrial cancer, and cardiovascular events, as reported from the Women's Health Initiative. Recent data demonstrating decreased coronary atherosclerosis in young postmenopausal women taking estrogen replacement provides additional reassurance [1].

Infertility is a significant issue for most women undergoing POF. Although many women will ovulate at some

point following the diagnosis and spontaneous pregnancies can occur in five to ten percent of those with idiopathic forms, this cannot be predicted with any reliability. There are many case reports and small series reporting use of various medical therapies in an attempt to restore ovarian function and fertility. Evidence suggests that pregnancies might occur if women with POF are managed to suppress their high FSH concentrations, either with ethinyloestradiol or with gonadotrophin-releasing hormone analogues, and then follow ovulation induction protocols using low-dose gonadotrophins [13]. However, randomized therapeutic trials fail to demonstrate any significant improvement in ovulation and pregnancy rates. Assisted reproductive technique with donated oocytes remains the only means for fertility treatment that carries high success rate. Cryopreserved embryos have also been employed for ovum donation in POF with a high pregnancy rate of 30% per transfer [8].

For most women, POF can be an unexpected and distressing diagnosis, with deleterious psychological impact, made worse by the fact that it coincides with infertility. Emotional support should be addressed to maintain their well-being. A positive and optimistic lifestyle should be encouraged to be maintained, including engaging in regular weight-bearing exercise, maintaining an adequate intake of calcium (1,200 mg daily) and vitamin D (at least 800 IU daily), and eating a healthy diet to avoid obesity. Regular screening for bone loss and cardiovascular risk factors is also recommended [13].

Conclusion

In conclusion, POF is a heterogeneous, multifactorial, and poorly understood condition that involves the psychological sphere and the sexuality of the affected patients. An early diagnosis and an immediate replacement treatment can prevent health associated problems, can relieve menopausal symptoms, and could provide considerable psychological support.

A genetic component reinforces the importance of a detailed family history. The difficulty to discover a determining factor should press to investigate other candidate genes through a gene mapping and establish their critical role in ovarian dysfunction. It is likely that this information may assist us to diagnose the condition earlier, and therefore provide a way to save or protect remaining good follicles before the development of POF. It is also possible that if a better understanding of the biology of POF is achieved, this may lead to possible therapeutic avenues for defective follicles to be developed further.

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Amniotic band syndrome (ABS): can something be done during pregnancy in African poor countries? Three cases and review of the literature

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Summary

Amniotic band syndrome (ABS) is a fetal congenital malformation, affecting mainly the limbs, but also the craniofacial area and internal organs. Two mains pathogenic mechanisms are proposed in its genesis. Firstly the early amnion rupture (exogenous theory) leading to fibrous bands, which wrap up the fetal body; secondly, the endogenous theory privileges vascular origin, mesoblastic strings not being a causal agent. The authors believe that the second theory explain the occurrence of ABS. The outcome of the disease during pregnancy depends on the gravity of the malformations. Interruption of the pregnancy is usually proposed when diagnosis of severe craniofacial and visceral abnormalities is confirmed. Whereas minor limb defects can be repaired with postnatal surgery. In case of an isolated amniotic band with a constricted limb, in utero lysis of the band can be considered to avoid a natural amputation. In an African country, such treatment is not possible as far as the antenatal diagnosis.

Key words: Amniotic band syndrome; Pathogenesis; Prenatal diagnosis and neonatal management; Fetal surgery; Doppler.

Introduction

Amniotic band syndrome (ABS) is a set of complex congenital malformations. They concern mainly limbs but also the craniofacial region and thoraco-abdominal axis. They are asymmetric, polymorphic, and have no embryological systematization [1]. If clinical signs are well known for many years [1, 2], the etiology and pathophysiology are always discussed [3-8]. B mode ultrasound (US) and color Doppler are essential in its antenatal diagnosis. In utero surgery in the treatment of amniotic band (AB) is an opportunity which then makes essential the understanding of its physiopathogenic process [9, 10]. It is however limited to developed countries where it is used everyday. In African poor regions, echography is not available in all the public maternities, as it is too expensive and therefore is inaccessible for the majority of pregnant woman.

The main objective of this work is to highlight the difficulties in an underdeveloped country, in the diagnosis and management of ABS, and their effects on the fetus. Thus, in three differing cases of ABS, the authors recall the epidemiology, clinical features, and prognosis of this disease. In a second step, they discuss the mechanisms of its supposed etiopathogenesis, its etiology, natural course, and therapeutic possibilities in their context.

Case Report

Case 1

The patient, 25-years-old, with no particular antecedent is followed in the present department for her fourth pregnancy (G4P3). US scan performed at 13 weeks of gestation (WG) was normal with a neck measuring 1.9 mm. Screening for trisomy 21 with serum markers of first trimester had not been performed. The routine US scan for fetal morphology was made at 22 WG. It showed anomalies of all the right hand fingers (syndactyly) except the thumb, associated to a voluminous edema of the right foot (Figures 1a, 1b). A second US scan was then requested for suspicion of ABS. It was taken three weeks later by another operator and confirmed abnormalities of the fingers of both hands and the right foot with the presence of amniotic band (Figures 2a, 2b). Fetoscopy in a reference center was then requested but refused by the family who asked for pregnancy medical interruption due to their small income, anguish, and anxiousness. The medical termination of pregnancy was obtained with prescription of oral prostaglandin (misoprostol). The macroscopic analysis confirmed the diagnosis by viewing a large amniotic string on the right foot with downstream huge edema of the instep and the sole. There were also upper limb disorders with syndactyly of the right hand (digits 2-3-4-5), a distal amputation, and syndactyly of the fingers of the left hand (digits 2-3-4) which preserved integrity of the thumb (Figures 3, 4). Pathological examination of the placenta and amnion confirmed the diagnosis of ABS.

Case 2

The patient, 35-years-old, without medical history, was in the fifth pregnancy of four living children. (G5P4) She was a housewife with low level of education as well as her husband. She was received for a systematic morphological US scan at 30 weeks. It showed an amputation of the right leg associated with edema of the left foot (Figure 5) and a clubfoot on the left. The fetal heart activity was normal and regular. Pregnancy was then carried to completion and childbirth was completed without major incident. Examination of the newborn found him in good health. The authors also noted the presence of an amputation of the right leg associated with club-foot (Figure 6) of left leg.

The newborn was then transferred to a neonatal orthopedic surgery where a splint was made for the foot. The output of the mother

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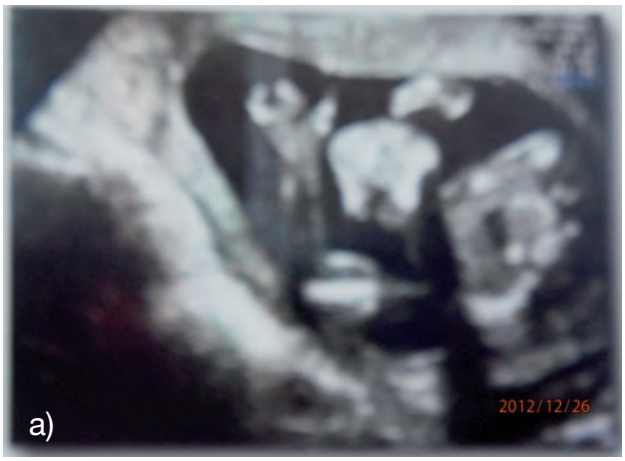


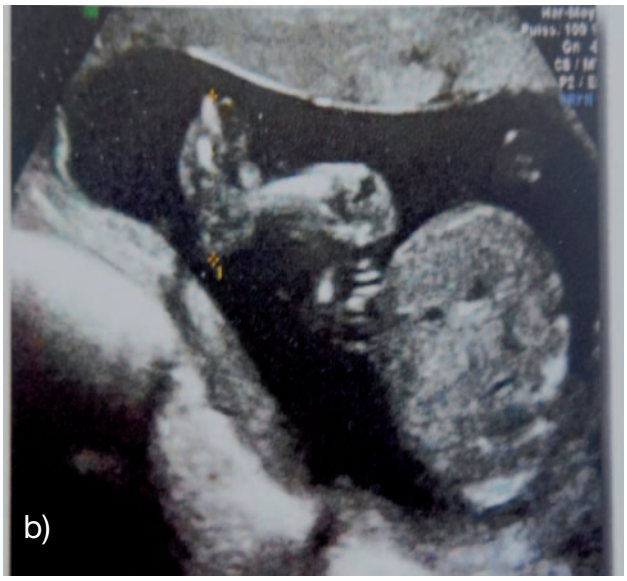
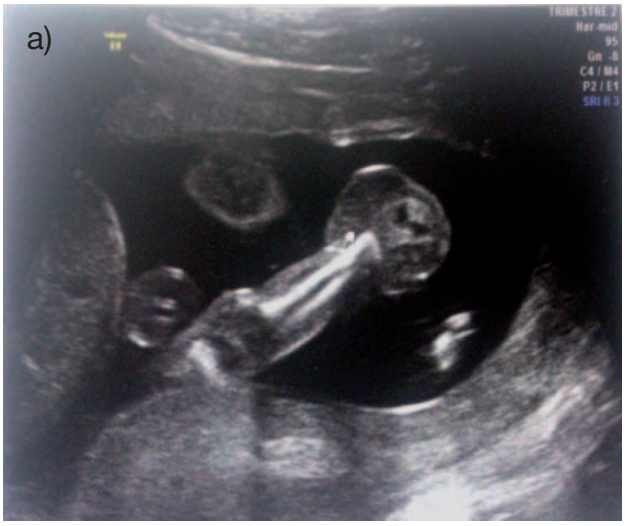


Figure 1. ·— Ultrasonography at 22 weeks gestation.
a) Right hand (syndactyly of digits 2-3-4-5.
b) Right foot edema.



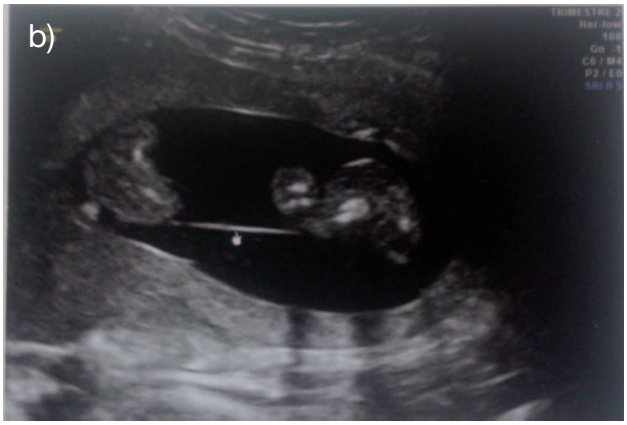


Figure 2. — Ultrasonography at 25 weeks gestation.
(a) Right foot large downstream edema
(b) Amniotic band.

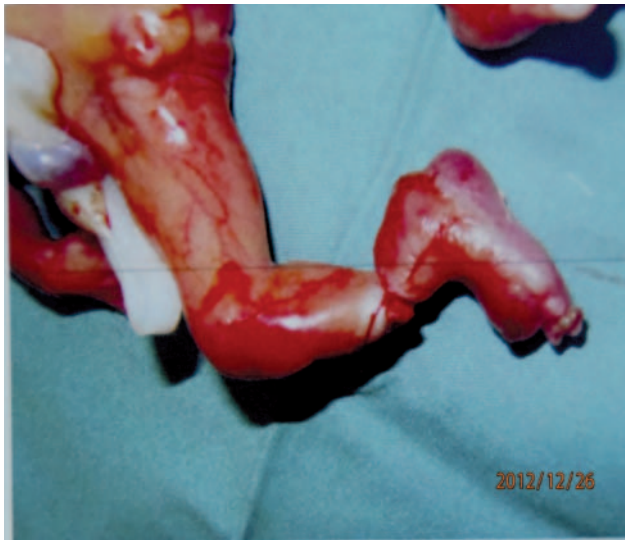


Figure 3. — Right foot large furrow constriction and downstream edema.

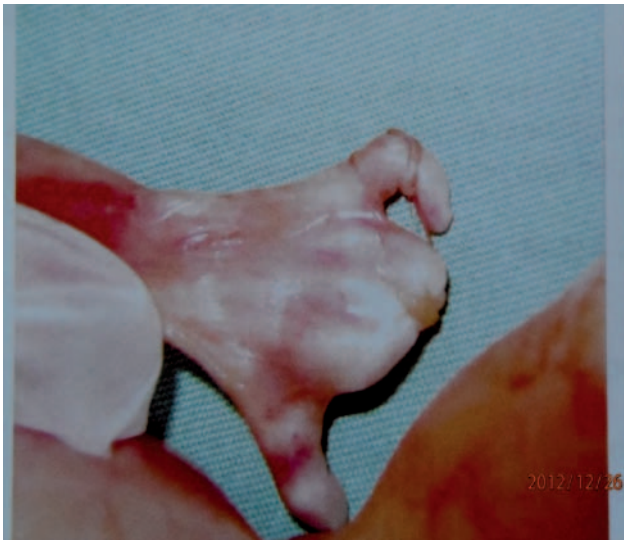


Figure 4. — Left hand syndactyly of digits 2-3-4.

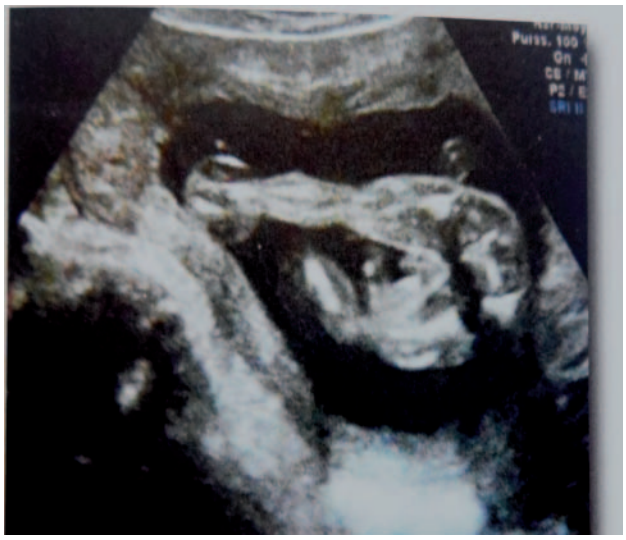


Figure 5. — Right leg amputation with edema of left foot at US.

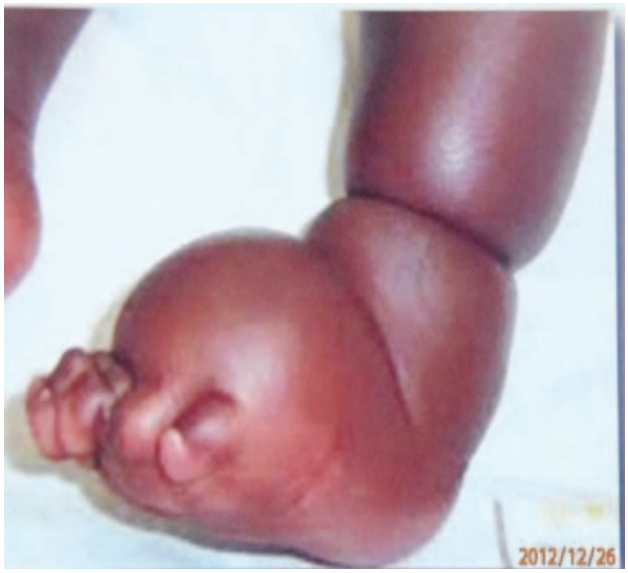


Figure 7. — Newborn at birth. Deep circumferential constriction band of right lower limb extremity associated with marked lymphedema and right clubfoot. No signs of soft-tissue necrosis or ulcerations are present.

was allowed 12 hours after birth in the absence of postpartum complications.

Case 3

The patients, 28-years-old, primiparous was received in emergency maternity for uterine contractions and loss of water on a term pregnancy. This pregnancy was characterized by poor monitoring because no prenatal diagnosis or prophylaxis had been performed. The examination on admission found woman at the end of the pregnancy term with imminent delivery. The prognosis of natural vaginal delivery was good, so the authors permitted it to continue. One hour later, she gave birth, without difficulty after perineum section, a newborn in apparent good health. Score Apgar 9 at third minute then 10 at 5 minutes. After delivery perineum

Figure 6. — Right leg amputation and left clubfoot.

suture the general examination of the newborn in the delivery room found at the left lower limb many cutaneous constriction furrows of the foot, with large downstream edema (Figure 7). The newborn was transferred to a neonatal orthopedic surgery for better decision support. The output of the mother was allowed 12 hours after delivery. Pathological examination of the placenta and amnion were not done to confirm diagnosis of ABS.

Discussion

Epidemiological factors

ABS is not frequent and its incidence is between one in 1,200 and one in 15,000 live births in developed countries [5]. Higher numbers of one in 55 to one in 250 [11], were recognized histological at examination of products miscarriages and reflect the lethal character of the syndrome, due to their incompatibility with life [2, 6, 8, 12, 13], or by strangling with the umbilical cord (UC) [5, 11, 14-17]. No racial predisposition or link to sex were found [18, 19]. Although a few familial cases have been described, the usually sporadic nature of this syndrome does not suspect a hereditary factor [19-23]. This frequency is underestimated in the underdeveloped countries as in Africa. Indeed, fetal pathological examination and statistics are not available. Moreover, antenatal US is not usually performed and most births take place in peripheric maternity hospitals, so the statistics are not available. These three reported cases were observed in the present maternity hospital and this is the reason why they could be reported.

Etiopathogenesis

Amniotic disease is a congenital disease whose etiology is unknown [24]. Despite numerous embryological, pathological, and experimental studies, there is no conviction about the origin or the ABS etiological factors [1]. Two opposing theories were found in literature to explain its genesis. The exogenous theory of Torpin [4] and the endogenous theory of Streeter [5].

According to Torpin's *exogenous theory* [5], the most commonly shared, the first factor was (oligoamnios) consecutive to breaking amnio-chorionic. It leads to an amnio-chorionic separation with leakage of amniotic fluid. Some anatomical parts of the fetus could then be partially exteriorized through the amniotic cavity, and thus become in close contact with chorion. The external surface of the amnion (mesodermic) then produces fibrous bands that surrounds and strangles them. This explains the phenomenon of constrictions and amputations [2, 5, 7]. AB is a very fibrous band of chorio-amniotic tissue which is often in contact with constriction skin furrows, digital amputations, pseudo-syndactyly, and facial clefts [25]. This is the common element that brought together this heterogeneity of the ABS. [25]. Fetus compression is secondary cause by oligohydramnios who plays a role in the genesis of certain malformations such as clubfoot [5, 26]. The severity of the malformation depends on the term of amniotic rupture [8, 27]. Craniofacial and visceral anomalies result from early rupture of membranes (28-45 days of gestation), whereas a constriction or amputation results from a late break (45 days to 18 weeks of gestation) [2]. Causal factors of early rupture of the amnion are not determined with certainty. The traumatic nature of amniocentesis is often reported as a possible cause of ABS [16, 20, 26, 28]. This theory, however, is deeply contested by many authors [4-9] because it does not explain the number and variety of malformations described [13, 29, 30]. In addition, for Bronshtein *et al.*, despite the multitude of US scans performed antenatally, no one has reported the following sequence: normal fetus –> membranes rupture –> amnion adhesion to fetus –> constriction or amputation [31]. In fact, he rarely found an AB in contact with an amputated limb segment when combining intrauterine AB and amputation segment. Other authors found no amniotic rupture in many fetuses with their anatomical elements in contact with an AB [8, 13]. Lastly, after amniotic membranes rupture, many extra-amniotic pregnancies were described without any AB or neonatal abnormality [32].

For the endogenous theory defended by Streeter, "there is no evidence demonstrating that intrauterine amputation was secondary to AB or an adhesive process or a constrictive process" [4]. An embryonic disc development anomaly, before the third week of gestation, is the cause of malformations as well as straps. The straps have no causal role [7, 16]. For Van Allen *et al.*, This anomaly would be responsible for infarction of embryonic vessels, causing tissue necrosis, cessation of embryonic development, hemorrhagic necrosis and the formation of AB by adhesion of the amnion to the necrosis parts [6]. Thus, the formation of straps could be compared to that of intraperitoneal adhesions after surgery [6]. To Hartwig *et al.*, this theory explains the variety of clinical manifestations of this disease, and in particular the internal organ attack [33]. Experimental works helped to support this theory, or at least the endogenous basic element. Glucose injection into uterine subsidiaries of pregnant rabbits causes constrictions and limb amputations associated with ABs [34]. Hemorrhagic extravasation superficial vessels of fetus can explain the pathogenesis. Finally, constitutional collagen anomaly [34], imperfect osteogenesis [35], and bullosa epidermolysis [36] could also be the cause of some ABS. Despite all these numerous works, none of them have demonstrated the two main theories. It is therefore highly likely that several non-exclusive mechanisms involved in the formation of different lesions of the disease [8]. In the present cases the authors believe that ABS seems more to be explained by the endogenous theory of Streeter, than that of Torpin [4, 5]. Indeed, lesions occurred when there were no abnormalities of amniotic fluid and the membranes were intact.

Clinical aspects

The ABS is a syndrome associated with multiple malformations, polymorphics, and asymmetrics without any embryonic systematizing [12]. Its severity is highly variable. The authors have found isolated skin furrow, craniofacial and visceral malformations, often incompatible with life [2-5]. The frequency of these malformations in the ABS is very variable according to some authors [3, 8, 13], but 77% of cases would at least present two anomalies [37]. Anomalies of limb are the most common, especially in patients with a single anomaly [2] (Figures 6, 7).

in furrows occur in decreasing order of frequency in the fingers, with relative sparing of the thumb (Figures 1a, and 5), toes, legs, forearms, hands, feet, arms, and legs [25]. Occurrence in the trunk is exceptional. Regarding amputations, they are asymmetric, often reaching the distal ends (Figure 6) and most often reaching the toes [39] (Figure 4). Amputation of an entire limb segment is uncommon [3]. The clubfoots, less specific abnormalities, would be a consequence of oligohydramnios (Figure 7) [1]. Craniofacial abnormalities affect especially the skull (anencephaly, or encephalocele). They have a lateralized character or anterior evocative without being pathognomonic [12]. Deformities such as facial clefts are possible [40]. Contrary to genetic malformations, these craniofacial anomalies are often asymmetric and outside embryonic development lines [25]. They are attributed to reach this region by AB which create an obstacle to the fusion of embryonic bud. Visceral malformations of thoraco-abdominal axis are more uncommon and never isolated. [2] They often correspond to closing parietal anomalies (gastroschisis and omphalocele). As regards the annexes, funiculars anomalies (short cord) are considered as classic AB, although highly controversial [11].

Prenatal diagnosis

US scan remains the main complementary investigation that can be coupled to fetoscopy. They are unavailable in our poor countries. For some authors, it allows the diagnosis of

some ABS in the first trimester, according to the nature and severity of malformations [27, 41]. Craniofacial defects and thoraco-abdominal can be detected from the first US scan at 10-12 WG. Isolated anomalies of limbs are generally diagnosed at a new ultrasound. In the present first observation, the diagnosis was made early. It was late or unknown in the other observations. In poor countries, rarity of the disease makes the training of sonographers difficult. Besides, the US machines are not high-performance. In fact, the prenatal diagnosis of AB is not essential to the diagnosis of ABS. It should however be considered in the presence of some characteristic signs such as asymmetric constriction or amputation of a limb with lymphedema end downstream of the constriction (Figure 7), a craniofacial malformation asymmetric (encephalocele, cleft lip, and palate); coelosomies the presence of pseudo-syndactyly or BA in contact with the injured fetal pole. All these lesions are accessible for trained sonographers with a high-performance machines.

Color and pulsed Doppler is also essential to confirm antenatal diagnosis of AB. It shows no vascularization in color and pulsed Doppler in the straps [42]. Most of the US scan machines used in the present country do not include color and pulse Doppler. Other associated signs may be find such as oligohydramnios, decreased fetal movement, and clubfoot that are a consequence of oligohydramnios [7, 8]. The study of umbilical Doppler could theoretically detect constrictions cord in utero. Color Doppler and pulsed of downstream limb of a constriction could be a key parameter to determine the natural history of the constriction. Tadmor *et al.* [41] have recently report the occurrence of in utero amputation secondary to constriction of a limb by an AB. There was a decrease and an absence of vascular flow, downstream of the constriction, the day before amputation [41]. In the present observations, this review has not been completed, due to its unavailability (observations 1 and 2), or late diagnosis at birth (observation 3). The study of Doppler strangled limb would assess the extent of the consequences of the constrictor ring to determine the natural history of the disease and inform parents of postnatal care. However, it is only an attractive hypothesis which must be demonstrated [1].

Radiography of the uterine contents can be use. Its diagnosis skeletal abnormalities (vertebral and limb deformities) and objectify the preservation of bone mineralization in the strangled limb. The karyotype is still regular. However, its realization seems justified in cases of diagnostic doubt to eliminate differential diagnoses. It is rare that it is done in the present context because it is too expensive. Histology examination remains indispensable in all cases. It confirms the diagnosis by showing the presence chorion separated partially or all of the amnion in front of an ABS [13]. It can also highlight areas of necrosis and remodeling of the amnion associated with proliferation of fibroblasts in the lamina propria, which may correspond to the stigma of a earlier rupture of the amnion [11].

Differential diagnosis

When craniofacial or limbs anomalies are accompanied of AB, there is little doubt about the diagnosis. However, some anomalies and syndromes, sometimes hereditary, have similar characteristics to ABS. [43] It may be an anencephaly due to primary defect closure of neural tube, facial clefts (lip and palate), Cantrell pentalogy [9], limb-body wall complex [8, 43], and congenital tegumentary aplasia [25]. We must also consider sub-chorionic hemorrhage [41], lack of fusion of the amnion [8], second twin evanescence [44], biamniotic twin pregnancy [43], bilobed placenta [44] or circumvallata [41].

Disease natural evolution

The natural evolution of ABS is unpredictable [11]. However, two parameters have not yet been sufficiently evaluated, could have a useful predictive value. It is either the occurrence of distal lymphedema reflecting constriction of neurovascular structures and lymphatics, or the study of vascular flow (color and pulsed Doppler) within the strangled limb which could be the most predictive parameter of eventual amputation.

Despite a regular ultrasound monitoring, it would be impossible to differentiate constrictor rings leading to a future in utero amputation (fetal indication for surgery) and those responsible for a isolated constriction skin furrow (indicating an attitude not interventionist). In the presence of craniofacial and visceral polymalformations, the natural history is of little importance, the prognosis is known and dark. The situation is quite different in the case of isolated and superficial constriction of a limb, as was the case in the present observations 2 and 3. Schematically, we can retain 4 scenarios. Firstly, exceptional spontaneous regression without after-effects of constriction [45]. Secondly, the limb constriction becomes deep, with neurovascular structures strangulation and cutaneous necrosis and subcutaneous risk downstream. Thirdly ring constrictor causes limb in utero amputation (observations 2 and 3). Finally, the occurrence of lymphedema downstream of a constriction is a result of vascular compression of arterio-venous structures and lymphatic fearing a decrease in vascular perfusion in the distal segment and amputation in utero [46].

Prognosis

Obstetrical prognosis of patients with abnormal fetuses associated with the ABS do not change compared to the general population [2, 3, 18]. The prognosis of surviving children is variable and depends on the severity of fetal injury. Survivors with severe craniofacial malformations number are few and then have motor, behavioral, and cognitive disorders [18]. A medical interruption of pregnancy may be proposed when the fetal abnormalities are recognized.

In the case of isolated anomaly of the extremities, the prognosis is excellent [2]. However, the prognosis of isolated constriction is closely correlated with the state of the

cutaneous tissue and subcutaneous downstream, as well as a possible infringement of neural structures [38]. No statistically significant cohort study has evaluated the functional outcome of a constriction according to its severity. However, it is estimated that postnatal surgery preserves functional capacity close to normal in 50% [44]. The psychological trauma felt at birth of such children and the more frequent proceedings, must lead us to a better diagnosis in front of such malformations. As long as the diagnosis is made, which is not always the case with our poor countries treatment must be proposed to the parents.

Treatment

Only isolated or associated anomalies must be concerned by treatment (furrows constriction, pseudo-syndactyly, and club-feet). The severe craniofacial and visceral polymalformations generally incompatible with life, are inaccessible to any therapeutic option. Without any in utero treatment (observations 2 and 3), skin creases and pinch pseudo-syndactyly are irreversible but do not worsen after birth [47]. In case of vascular impact (venous stasis and lymphedema), excision with release of neurovascular bundle is urgent and performed within the first 48 hours of life. In front of constrictive circumferential furrows, microsurgical excision in one time gives a better cosmetic result [48]. Instituted edema does not regress. If the constricting furrow is very tight, the skeletal lesions with thinning of bones or multiple fracture can be observed [49]. Pseudo-syndactyly will be released early in the neonatal period, to limit the movement towards camptodactyly. When the end of the two fingers is connected by a single membrane, it may simply be excised and sutured. The most important mergers, will be associated with dissection of a skin graft to prevent adhesions during healing [25, 47].

In the case of digital amputation, a secondary release can be performed to give the maximum length to amputated fingers [49]. The clubfeet with a knee furrow, respond less well to orthopedic treatment than idiopathic idiopathic. To avoid the occurrence of in utero amputation, some authors have suggested in utero release of constricting ring. This was the case of Quintero *et al.* [10] in 1997 that released in utero constrictor amniotic ring under US and endoscopy. Since no other similar experience has been reported in the literature. In fact, in utero treatment of isolated constriction of limb is controversial. Indeed, the natural evolution of a limb constriction remains unpredictable. There is currently no indication for in utero surgery in the ABS. Many authors dispute the fact that in utero amputation is linked to the hypothetical process of constrictive AB [6, 9, 11]. In addition, this surgical procedure is not simple nor free of complications. Finally, infants with a superficial skin furrows have an excellent functional prognosis, in contrast to those with neurovascular structures' constriction [1]. Beyond 32 weeks, fetal extraction could be an interesting compromise between the functional prognosis and prematurity, and an alternative to in utero surgery. Nonetheless, before considering in utero treatment, it is essential to ensure the absence of other malformations. In the present second observation, the expectation was observed due to our inability to perform in utero surgery.

Conclusion

ABS is an embryo-fetopathy acquired with a set of asymmetric malformations, primarily in the limbs. Although its pathogenesis remains controversial, it probably results due to many different pathological processes. Therefore in poor Africans countries were illiteracy is higher and incomes of population lower, the support of ABS always remains difficult and fetal prognosis is dismal. More monitoring of pregnancies and in utero surgery must be practiced more frequently in African countries to improve it.

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Partial agenesis of corpus callosum - case study

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Summary

Agenesis of the corpus callosum is an uncommon cerebral malformation usually of unknown etiology. It can be associated with other brain abnormalities, such as ventriculomegaly, or in combination with problems with other organs, such as congenital heart defect, as well as with chromosome anomalies. Diagnosis of this rare anomaly is important not only because of possible association with other developmental anomalies but also because of postnatal treatment and evaluation of children with this disorder. This paper presents prenatal diagnosis of partial agenesis of the posterior part of corpus callosum of a fetus detected in gestational week 33 by ultrasonography as an isolated developmental disorder, i.e., not accompanied by other morphological anomalies of the fetus or chromosome aberrations or other genetic defects.

Key words: Fetal corpus callosum; Corpus callosum agenesis.

Introduction

The corpus callosum is a collection of many millions of nerve fibers in the middle of the brain. One of its functions is to connect the right and left side of the brain to allow for communication between the two sides, or hemispheres. The corpus callosum coordinates signals from different parts of the brain and helps the thinking process.

Agenesis of the corpus callosum is a birth defect in which this structure in the brain is either partially or completely missing. It may occur as an isolated brain problem (49% of cases), in combination with other brain abnormalities, such as ventriculomegaly, or in combination with problems with other organs, such as congenital heart defect. In addition to agenesis of the corpus callosum, other callosal disorders include hypogenesis (partial formation), dysgenesis (malformation) of the corpus callosum, and hypoplasia (underdevelopment) of the corpus callosum. Agenesis of the corpus callosum is caused by disruption during development of the fetal brain between the third and 12th week of pregnancy but it is diagnosed considerably later, usually after 30th week of gestation. In most cases, it is not possible to know what caused this anomaly. However, research suggests that some possible causes may include chromosome errors (most frequently trisomy 13 and 18), inherited genetic factors, prenatal infections or injuries, prenatal toxic exposures, structural blockage by cysts or other brain abnormalities, and metabolic disorders [1].

Callosal disorders can be diagnosed only through a brain scan. They may be diagnosed through magnetic resonance imaging (MRI), computed tomography (CT) scan, prenatal ultrasound or prenatal MRI. Prenatal diagnosis of this malformation is now routinely performed by ultrasonography (after 20th week) and MRI (after 30th week).

Agenesis of the corpus callosum is an uncommon cerebral malformation that has been reported in one in 19,000 unselected autopsies and 2.3% of children with mental retardation [2].

Partial agenesis of the corpus callosum is a very rare birth defect.

Case Report

Case of partial agenesis of corpus callosum in a fetus from a pregnancy detected through in vitro fertilization (IVF) program is presented. Anomaly was detected by ultrasonographic exam in gestational week 33 and subsequently confirmed by fetal MRI two weeks later.

IVF was performed due to male sterility i.e. very poor spermogram results. Pregnancy was achieved at 34 years of age of both partners, whose findings were all (except the spermogram in male partner) normal. In gestational week 12,6 a biochemical screening was performed for chromosomal anomalies. Finding was normal. Prenatal invasive diagnostics (chorionic villus sampling, amniocentesis) was not performed. Fetal growth and development were subjected to regular ultrasonographic checkups, fetal morphology was normal, biometric parameters corresponded to gestational age until week 32. In 33rd week of gestation the ultrasonographic exam of fetal head showed a deviation-biparietal diameter (BPD) corresponded to 36 weeks instead of 32,3 gestational weeks (Figure 1), together with discrete widening posterior cornu of the lateral ventricles up to 12 mm.

A 3D/4D multislice ultrasonographic examination of the fetal brain was performed. Cavum septi pellucidi was slightly narrowed and irregular in shape, lateral ventricles borderline expanded, while the posterior part of corpus callosum failed to be visualized. Doubt was raised concerning partial agenesis of the corpus callosum (Figure 2). Patient was referred to fetal endocranial MRI exam which confirmed the absence of the posterior part of the corpus callosum, i.e. partial agenesis of fetal corpus callosum.

Considering that the defect was very small, located in the posterior third of corpus callosum, and that there were no other visible anomalies, pregnancy was carried to term. Elective cesarean section was performed at term and a healthy male child weigh-

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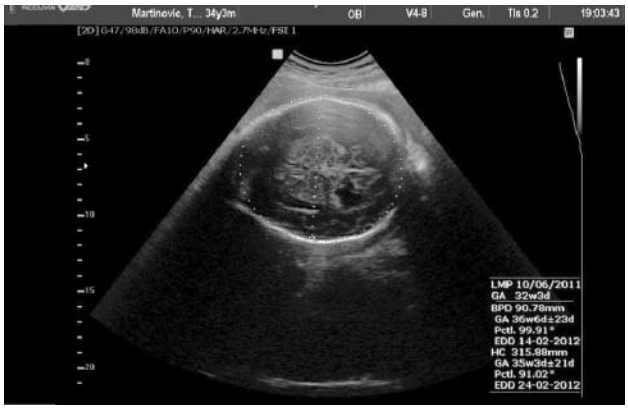

Figure 1. — Biparietal diametar of the fetal head.

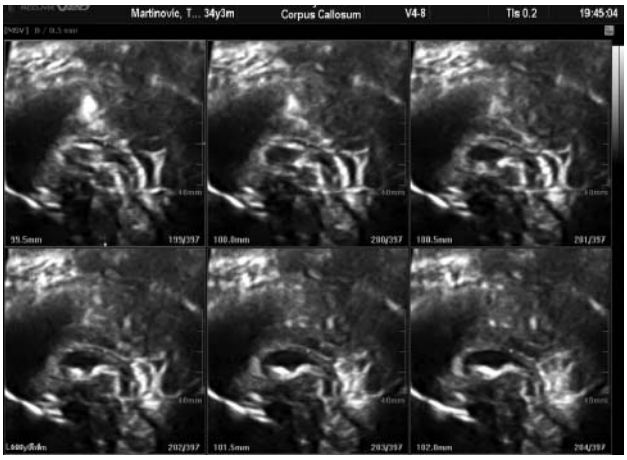

Figure 2. — 3D/4D multislice ultrasound of fetal brain.

ing 3,600 grams was born. Several ultrasonographic exams of the fetal brain were performed in the early neonatal period (Figure 3). Existence of a minute defect in the posterior part of corpus callosum was confirmed, calling for nuclear MRI which was in compliance with ultrafonographic exams. Regular monthly checkups were performed during the first six months, followed by increase of interval to two months over the second six months. Fetal brain development was normal while previously visualized defect did not show any increase. Growth and development of the fetus were normal and no disorders in mental functions were noted during the first years of life.

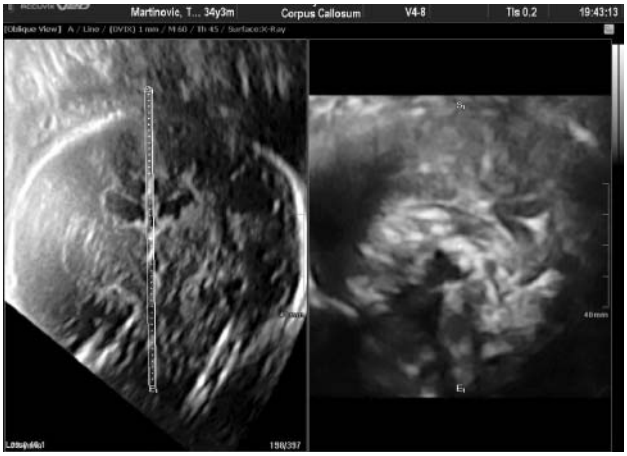

Figure 3. — Ultrasound scan of fetal brain.

Discussion

Several studies from literature analyze the disorders in development of corpus callosum, complete absence of this brain structure i.e., complete agenesis; these studies date from 1990s until today. Majority of described cases were detected between 19 and 37 weeks of gestation. The research of Pilu *et al.*, dating from 1993, for example, showed 35 fetuses in which ultrasonographic imaging detected absence of the corpus callosum and cavum septum pelucidum with typical "teardrop" configuration of the lateral ventricles, distension of the interhemispheric fissure, upward displacement of the third ventricle, radiate arrangement of the medial cerebral gyri, and abnormal branching of the anterior cerebral artery [3]. Narrowing of the corpus callosum and the so-called "teardrop" configuration of the lateral ventricles was also verified in the presented case.

Establishing the reference range during human pregnancy for normal fetal corpus callosum is especially important for the detection of this rare anomaly. The research of Professor Achiron, dating back to 2001, for example, defines criteria for fetal corpus callosum measurement in the period from 16th to 37th week of gestation, follows growth and development of corpus callosum (by weekly ultrasonographic measurements), with the aim of defining criteria for size of corpus callosum measured in all three sections in each week of gestation. This study offers normative measurements of the fetal corpus callosum and may facilitate a more objective diagnosis of its congenital abnormalities [4].

Research performed by d'Ercole *et al.* in 1998 compares the accuracy of ultrasonographic diagnostic of defects in development of corpus callosum with that of MRI diagnostics performed after this development defect was suspected during ultrasonographic exam [5]. This research showed that ultrasonography was able to suspect agenesis of the corpus callosum by indirect signs but definitive diagnosis of corpus callosum agenesis was achieved in only four of 14 cases.

Application of 3D/4D multislice ultrasonographic exams significantly increased diagnostic accuracy in detecting this rare anomaly in brain development; this is confirmed in the case described in the present study. The corpus callosum can be assessed on ultrasound by direct visualization, but indirect features, such as ventriculomegaly, absence of the cavum septi pellucidi or widening of interhemispheric fissure, are often reasons for

detection in a screening population. Careful imaging in center with a high level of expertise is required to make a full assessment and to exclude coexisting abnormalities, which occur in about 46% of fetuses [6].

Even though partial agenesis of the corpus callosum is fortunately not a frequent anomaly, it is necessary to consider the possibility of occurrence of this disorder in development of brain structures, not only because it may be associated with other developmental anomalies, but also because of the postnatal treatment and follow-up of children diagnosed with it.

There are currently no specific medical treatments for callosal disorders, but individuals with agenesis of the corpus callosum and other callosal disorders may benefit from a range of development therapies, additional support, and services. It is important to consult with a variety of medical, health, educational, and social work professionals. Such professionals include neurologists, neuropsychologists, occupational therapists, physical therapists, speech and language pathologists, pediatricians, music therapists, geneticists, special educators, early childhood intervention specialists, and caregivers for adults.

Research performed by Moutard *et al.* in 2012 analyzes development of children prenatally diagnosed with corpus callosum agenesis over a ten-year period and concludes that although prenatal diagnosis of isolated corpus callosum agenesis is reliable, false postnatal diagnoses remain possible (10-20%) even with complete prenatal screening. Outcome is mostly favorable because intelligence is within the normal range for nearly 3/4 of children. However, they frequently have mild learning difficulties [7].

The prognosis in cases of isolated agenesis of the corpus callosum remains uncertain, although it is expected that a normal or borderline intellectual development will occur in many cases.

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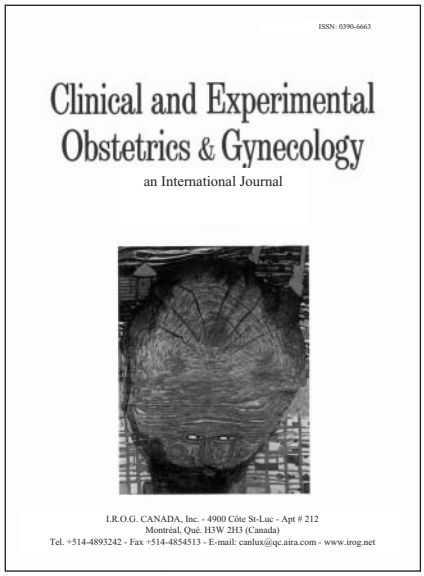

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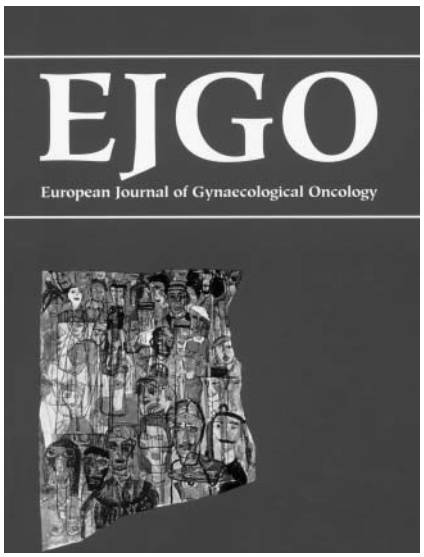

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