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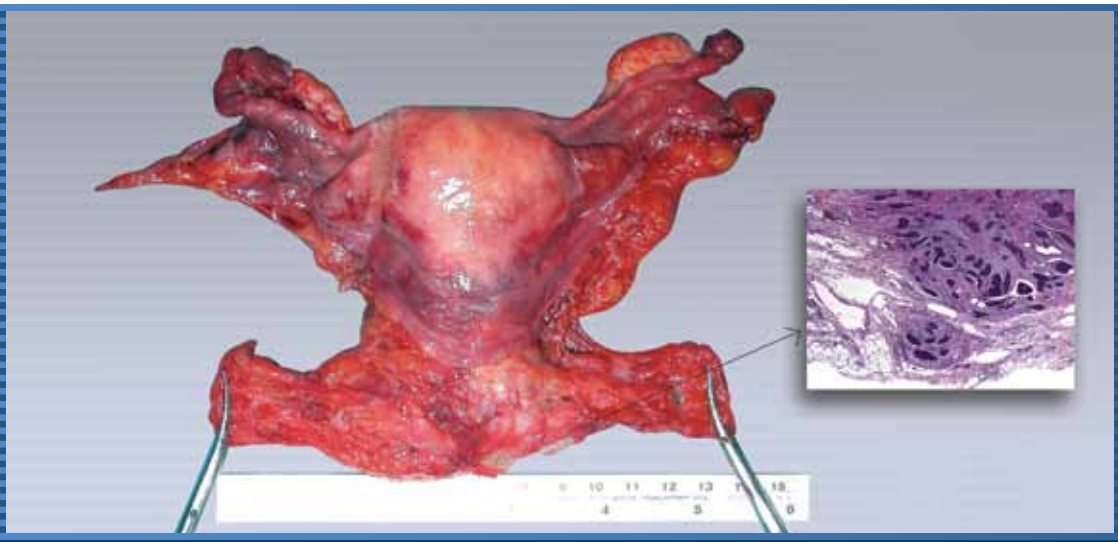

"Pathological Specimen after Radical Operation"
The courtesy of Ali Ayhan.

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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December.

The target audience of Journal of the Turkish-German Gynecological Association includes gynaecologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

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The "Journal of the Turkish German Gynecological Association" (ISSN 1309-0399; Abbreviated as "J Turkish German Gynecol Assoc") is the official publication of the Turkish-German Gynecological Education and Research Foundation and the Turkish-German Gynecological Association. Formerly named "ARTEMIS" is printed quarterly (March, June, September, December) and publishes original peer-reviewed articles, reviews, case reports, brief reports and commentaries in the fields of Gynecology, Gynecologic Oncology, Endocrinology & Reproductive Medicine and Obstetrics in English. The title, abstract, and key words (according to medical subject headings) are provided in English at the beginning of each article. Reviews will be considered for publication only if they are written by authors who have at least three published manuscripts in the international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

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Contents

Journal of the
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Editorial

Dear Colleagues,

I am proud to present you the second issue of Volume-14 of JTGGA with plenty of meritorious scientific studies. Our objective is to collect more and more research studies from Turkey and the international gynecology and obstetrics community.

In this particular issue, you will find a chance to read an interesting paper that demonstrates the histopathological basis of individually tailored less radical surgery concept in patients with International Federation of Gynecology and Obstetrics (FIGO) stage IB cervical cancer by an esteemed author - Ali Ayhan, and his team. Nowadays, the intention of the hysterectomies is rapidly turning to minimally invasive approaches. You may also find a nice study that evaluates the effects of previous abdominal surgery on the feasibility of operation and the safety of total laparoscopic hysterectomy. Over and above, a few fine experimental studies are also published in this issue. One of them is about melatonin and its therapeutic effect on the rat endometriosis whereas the other one is about the effects of lipoid acid in the prevention of postoperative pelvic adhesions by a visual scoring system and immunohistochemistry in a rat uterine horn model.

Everyone shall agree that the power of the meta-analysis is crucial. There are two meta-analyses studies in this June issue. One of them is about 'Recurrent Endometriosis', and the other one is about 'Pregnancy of Unknown Location'. Besides, four interesting case reports and an informative quiz will attract your interest in the following pages of the journal.

I would proudly like to inform you about the **2nd International Research Awards on Obstetrics & Gynecology**, which will be granted to three researchers or research groups who have been able to carry out the best researches in the field of Obstetrics & Gynecology or related subjects and submit them to the Journal of Turkish German Gynecology Association (JTGGA) by the online submission system at www.jtgga.org. Best three abstracts will also be awarded financially, as the best one with **$ 5.000**, second one with **$ 3.000** and the third one with **$ 1.500**.

The aim of this award is to appreciate the prolificacy of our colleagues in research projects and to encourage especially our young colleagues for the forthcoming years. The manuscripts submitted for 2nd International Research Awards will be peer-reviewed in terms of their scientific contribution, originality, content and the accepted manuscripts will be published at the JTGGA. Deadline for Manuscript Submissions will be **February 21st, 2014**. The award winners will be announced at the first issue of the year 2014 of the JTGGA and the award will be presented at the Opening Ceremony of the X. Turkish German Gynecology Congress. The corresponding author of the Best Manuscript will also be awarded with a free **Registration and Accommodation Package** for the X. Turkish German Gynecology Congress and an **Oral Presentation** about the Best Manuscript will be allocated in the scientific program.

We are working hard for the organization of the next congress of our foundation - X. Turkish German Gynecology Congress that will be held between the dates of April 30th and May 4th, 2014 in Antalya.

It is a common wish that our congress bringing together many of our colleagues nationally and internationally since 1995, will find rather a lot of **twentieth** anniversaries. I would like to remind you to mark **April 30th, 2014** on your calendars.

I wish all of you, working hard for a long time, a beautiful summer holiday with a plenty of sun.

Best regards,

Prof. Dr. Cihat Ünlü
Editor in Chief of JTGGA
President of TAJEV

To what extent should we perform parametrectomy in FIGO stage IB cervical cancer?

FIGO evre IB serviks kanseri olgularında parametrektominin sınırları ne olmalıdır?

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Abstract

Objective: To demonstrate the histopathological basis of individually tailored less radical surgery concept in patients with International Federation of Gynecology and Obstetrics (FIGO) stage IB cervical cancer.

Material and Methods: The study was performed in the gynaecologic oncology department of Başkent School of Medicine Hospital, Ankara, Turkey between January 1st 2008 and January 1st 2009. Cardinal and uterosacral ligaments were serially sectioned in an alternative approach, and were examined by a senior gynaecologic pathologist. Clinical and pathological features and findings in sections of uterine ligaments were recorded. Study data were analysed using the SPSS 17.0 program.

Results: Thirty-two out of 38 cases had squamous cell carcinoma (SCC) (84.2 %), and six had non-squamous cell tumours (15.8%). Four cases had microscopic (10.5 %) and one case had macroscopic (2.6 %) tumour extension in cardinal ligaments. Mean tumour-free cardinal ligament length was 16.8±7.39 mm. Presence of tumour invasion in cardinal ligaments correlated significantly with pelvic lymph node metastasis (p=0.02). No isolated tumour deposits were found in any of the cases in serial sections of the cardinal or uterosacral ligaments.

Conclusion: This research was designed as a preliminary study. Future studies are needed to determine the optimal resection margins of the uterine ligaments in surgically treated stage IB cervical cancer. With continuing research and the development of newer surgical techniques, patients' quality of life will be optimised accordingly.

(J Turkish-German Gynecol Assoc 2013; 14: 63-7)

Key words: Cervical cancer, radical hysterectomy, parametrectomy, histopathology

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Özet

Amaç: Uluslararası Jinekoloji ve Obstetrik Federasyonu (FIGO) evre IB serviks kanseri olgularında hastaya özel belirlenen, daha az radikal cerrahi yaklaşımının histopatolojik temellerini ortaya koymaktır.

Gereç ve Yöntemler: Çalışma Başkent Üniversitesi Tıp Fakültesi Jinekolojik Onkoloji bilim dalında 1 Ocak 2008 ile 1 Ocak 2009 tarihleri arasında yürütülmüştür. Tip III histerektomi cerrahi spesimenlerindeki kardinal ve uterosakral ligamentler seri kesitler alınarak deneyimli bir jinekopatolog tarafından incelenmiştir. Olguların klinik ve patolojik verilerinin yanısıra, uterin ligamentlerin seri kesitlerindeki bulgular ortaya konulmuştur. Çalışma verilerinin istatistiksel analizi SPSS 17.0 programı ile yapılmıştır.

Bulgular: Çalışmaya dahil edilen 38 hastanın 32'sinde (%84.2) skuamoz hücreli, geriye kalan 6'sında (%15.8) skuamoz hücre dışı tümörler mevcuttu. Dört olguda (%10.5) mikroskopik, bir olguda (%2.6) da makroskopik olarak kardinal ligament tutulumu mevcuttu. Ortalama tümörsüz kardinal ligament uzunluğu 16.8±7.39 mm idi. Kardinal ligamentlerde tümöral tutulum olması ile pelvik lenf nodu metastazı arasında anlamlı bir ilişki mevcuttu. Kardinal ve uterosakral ligamentlerin seri kesitlerinin hiçbirinde izole tümör depozitlerine rastlanmadı.

Sonuç: Bu araştırma, öncü bir çalışma olarak dizayn edildi. Cerrahi olarak tedavi edilen FIGO evre IB serviks kanserli hastalarda uterin ligamentlerin optimal rezeksiyon sınırlarının belirlenebilmesi için gelecek çalışmalara ihtiyaç vardır. Süren araştırmalar ve yeni cerrahi tekniklerin gelişmelerinin eşliğinde, hastaların yaşam kaliteleri de optimize edilecektir. (J Turkish-German Gynecol Assoc 2013; 14: 63-7)

Anahtar kelimeler: Serviks kanseri, radikal histerektomi, parametrektomi, histopatoloji

Geliş Tarihi: 27 Mart 2013 **Kabul Tarihi:** 10 Nisan 2013

Introduction

Radical abdominal hysterectomy (RAH) has been the traditional treatment in most cases of early stage cervical cancer (CC) for over a hundred years (1). In the class III RAH procedure, along with excision of the uterus, uterosacral ligaments are divided at their origin close to the sacrum, and cardinal ligaments are divided at the pelvic side-walls (2). Although extensive resection of the parametrial tissue ensures the highest probability of tumour removal, it is worth noting that these procedures carry a substantial risk for many subsequent complications, which are predominantly of genitourinary and gastrointestinal origin. Sexual dysfunction is also not uncommon (3).

Considering the high survival rate of appropriately treated early stage CCs, it cannot be overemphasised that every effort should be made to avoid post-operative long term sequelae (3). The morbidities arising after extensive procedures have led us to search for ways to limit "radicality" without jeopardising oncological outcomes. In recent years, a remarkable trend towards less radical surgery in CC treatment has been observed in the literature. With the patients' quality of life (QOL) on focus, many investigators in the field have proposed various surgical techniques to lower mor-

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bidity (4-8). Moreover, various surgical classification systems have recently been proposed, which include nerve-sparing procedures (9, 10).

In this study, we examined the cardinal and uterosacral ligaments of specimens from class III radical hysterectomy procedures, all of which were performed for International Federation of Gynaecology and Obstetrics (FIGO) stage IB cervical cancer. Tissue analyses were performed thoroughly, utilising an alternative sectioning method. We searched for signs of isolated tumour involvement, and aimed to demonstrate the histopathological basis of currently evolving "less radical surgery" concept.

Material and Methods

The study was performed at the Gynaecological Oncology Department of Başkent University School of Medicine, between January 1st 2008 and January 1st 2009. Scientific and ethical approval was obtained from the institutional review board (KA11/08). The study was funded by Başkent University School of Medicine. Thirty-eight surgically treated cases of FIGO stage IB CC were prospectively included in the study. All procedures were performed by the same surgical team. In all of the procedures, bilateral pelvic and para-aortic lymph node dissection (LND) preceded class III RAH, excising the cardinal ligaments close to the pelvic sidewalls, and uterosacral ligaments close to the sacrum. Bilateral uterine arteries were ligated at their origin on the hypogastric vessels.

Operative specimens were immediately placed in a formaldehyde solution in the operating room, and were taken to the pathology laboratory. Conventional gross and histopathological examinations were performed initially. Histological type and dimensions of the tumour, depth of stromal invasion, lymphovascular space involvement (LVSI), microscopic parametrial and vaginal extension status and tumour-free parametrial length were recorded. Regional lymph nodes were examined for tumour involvement. The excised cardinal and uterosacral ligaments were serially divided into 3 mm sections from distal to proximal, perpendicular to the long axis of the ligaments. The slides were numbered and dyed with haematoxylin and eosin (H&E) stain, and were examined by a senior gynaecological pathologist for any signs of tumour involvement. The study cases were subdivided into two groups with respect to tumour size: Group 1 for tumours <4 cm and Group 2 for tumours ≥4 cm. Evaluation of all cases together was followed by analysis according to groups.

Study data were analysed using SPSS version 17.0 statistical package program (Statistical Package for the Social Sciences, version 17.0, SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed by using Pearson's chi-square or Fisher's exact test, Student's *t* test and Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

Results

A total of 38 cases were included in the study. Twenty-five cases were in Group 1 and 13 cases were in Group 2. Mean patient age was 53.9±15.6 (range 28-87). Thirty-two out of the 38 cases had squamous cell carcinoma (SCC) (84.2%), and 6 had non-squamous cell tumours (15.8%). Four of the non-squamous group had adenocarcinoma and 2 had undifferentiated carcinoma. Mean tumour diameter was 3.2±1.7 cm (range 1.0-6 cm). Twelve cases (31.6%) had pelvic or para-aortic lymph node metastasis. Perineural invasion was present in 5 cases (13.1%). Clinical and pathological features of the cases are summarised in Table 1.

Four cases had microscopic (10.5%) and one case (2.6%) had macroscopic tumour extension in cardinal ligaments (Figure 1). Data regarding parametrial involvement is presented in Table 2. The presence of tumour invasion in cardinal ligaments correlated significantly with pelvic lymph node metastasis (p=0.02). Mean tumour size was higher in cases with parametrial involvement (p=0.03). However, the parametrial involvement rate was comparable between Group 1 and Group 2 (p>0.05).

The serial sections of cardinal ligaments were free of tumour deposits. Likewise, no tumour deposits were found in any of the cases in serial sections of the uterosacral ligaments.

Discussion

The oncological outcome of CC is generally favourable when diagnosed and treated at an early stage. However, many morbidities may arise after treatment, which ultimately result in the impairment of QOL (3). A number of therapeutic modalities have been suggested to prevent these undesired consequences of treatment (4-7, 11, 12). Class II RAH instead of class III was proposed for the treatment of early stage CC nearly two decades ago (13). The authors claimed that excellent cancer control with less morbidity was possible in class II RAH. Since then, researchers have focused on seeking the optimal surgical technique in CC treatment. In the trial of Landoni et al. (14), 243 patients with Stage IB1–IIA CC were randomised to receive either class II or class III hysterectomy. Both groups had similar recurrence-free and overall survival rates, but there was less morbidity seen in the class II group. A study performed by Cai et al. (15) also favoured class II RAH rather than class III in grade I and II cervical SCCs smaller than 2 cm. The authors reported similar oncological outcomes in the class II group, with significantly lower operating time, post-operative length of hospital stay, and estimated blood loss (15). It is worth noting that these two studies did not include stage IB2 cases. Individually tailored therapy for CC has also been defined, which deserves further evaluation (4, 16). A recently published study compared class I (extrafascial) hysterectomy versus class III RAH in stage IB1- IIA CC (8). Strikingly, there were no significant differences in recurrence rate and overall survival among extrafascial hysterectomy and class III RAH groups. Schmeler et al. (17) also published an extensive review on the conservative management of early stage cervical cancer. The authors focused on the current evidence that, in the presence of favourable pathological characteristics, a very low number of patients had parametrial tumour involvement. They concluded that, with sound data obtained from currently on-going studies, conservative treatment could be the standard approach in early stage CCs.

Table 1. Clinicopathological data of study cases

Parameter	All cases n:38 (%)[1]	Group 1 n:25 (%)[1]	Group 2 n:13 (%)[1]	p[2]
Mean age (±SD)	53.9±15.6	57±16.9	47.7±10.9	0.08
Mean tumour diameter in cm (±SD)	3.2±1.7	2.1±0.9	5.1±0.8	<0.001
Surgery				
- RAH + RLND	11 (28.9)	5 (20)	6 (46.2)	0.13
- RAH + RLND + BSO	27 (71.1)	20 (80)	7 (53.8)	
Histological Type				
- Squamous	32 (84.2)	20 (80)	12 (92.3)	0.64
- Non-squamous	6 (15.8)	5 (20)	1 (7.7)	
Primary Tumour Size				
- <4 cm	25 (65.8)	25 (100)	0	
- ≥4 cm	13 (34.2)	0	13 (100)	
Parametrial Involvement				
- Present	5 (13.2)	23 (92)	10 (76.9)	0.31
- Absent	33 (86.8)	2 (8)	3 (23.1)	
Lymph Node Metastasis				
- Absent	26 (68.4)	19 (76)	7 (53.8)	
- Only Pelvic	11 (28.9)	6 (24)	5 (38.4)	0.27
- Pelvic + Paraaortic	1 (2.6)	0	1 (7.8)	
LVSI				
- Absent	18 (47.4)	17 (68)	3 (23.1)	0.01
- Present	20 (52.6)	8 (32)	10 (76.9)	

[1]Column percentage
[2]Comparison between Group 1 and Group 2
*RAH: radical abdominal hysterectomy; RLND: Regional lymph node dissection; BSO: Bilateral salpingo-oophorectomy; LVSI: Lymphovascular space involvement

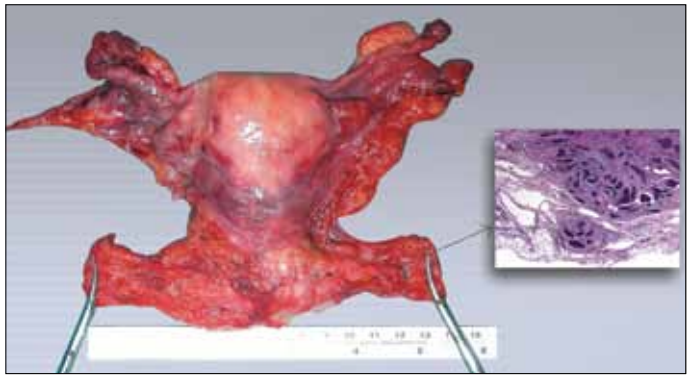

Figure 1. Pathological specimen after radical operation

In this study, we intended to demonstrate the histopathological findings of the major uterine ligaments comprising the parametrium of thirty-eight cases with FIGO stage IB CC. To the best of our knowledge, this is one of the few studies reported recently that investigate uterine ligaments thoroughly in surgically treated CC. Thus, it may serve as a preliminary report to guide future studies. All of the study cases underwent class III radical hysterectomy and pelvic-paraaortic LND. To obtain accurate results according to tumour size, we divided the cases into two groups and carried out analyses accordingly. It was interesting that there was no statistically significant difference between Group 1 and Group 2 regarding pelvic-paraaortic lymph node metastasis and parametrial involvement frequencies. However, mean tumour diameter was larger in cases with parametrial involvement. Although primary surgery is controversial in cases with tumours larger than 4 cm, the maximum tumour size in our subset of cases (Group 2) was 6 cm and the patients preferred primary surgery. We purposefully included these cases in the study to provide information on larger-sized tumours.

In our study, we could not demonstrate any sign of isolated tumour spread or lateral segment involvement of cardinal ligaments in any of the cases. One of the cases had 1 cm macroscopic cardinal ligament invasion, which was missed on preoperative evaluation but was evident on intraoperative exploration. The cardinal ligaments in that case were extensively excised, leaving at least 1 cm of tumour-free parametrium. A previous article by Hoffman et al. (18) reported the findings in completely excised vascular segments of radical hysterectomy

Table 2. Data regarding parametrial involvement

Parameter n:38 (%)a	All cases n:25 (%) a	Group 1 n:13 (%) a	Group 2	p*
Parametrial involvement				
- Absent	33 (86.8)	23 (92)	10 (76.9)	0.31
- Microscopic	4 (10.5)	2 (8)	2 (15.4)	
- Macroscopic	1 (2.6)	0	1 (7.7)	
Parametrial invasion depth (mm)	4.28±3.28	2.6±0.5	5.4±4.0	0.35
Tumour-free parametrium (mm)	16.8±7.4	10.5±0.7	21.0±6.5	0.10
*Comparison between Group 1 and Group 2				

specimens. In this study, 1 case was FIGO stage IA2, 45 cases were stage IB1, 31 cases were stage IB2 and 7 cases were stage IIA. In 84 cases, 19 women had nodal (parametrial lymph nodes) or non-nodal (LVSI or discontinuous tumour cells) vascular segment disease, of which 7 were in the lateral segment. The authors concluded that, although there may be subgroup of CC cases with isolated medial parametrial metastasis that are appropriate for modified parametrial resection, cases with preoperative high risk features (large tumour, vaginal involvement, extensive LVSI, deep stromal invasion) should be treated by complete resection of the vascular portion of the cardinal ligament and LND. When the cardinal ligaments are predominantly involved in cervical tumour extension, it is worth remembering that these ligaments constitute the main route of CC spread, primarily via lymphatic circulation (19, 20). Although there are aforementioned publications that report equivalent therapeutic results in both class II and class III radical hysterectomies in early stage CCs, the probability of leaving residual disease should always be kept in mind (18). Many authors recommend at least 1 cm of tumour-free resection margins, where possible. However, the safest margin of resection needs to be defined by future studies.

There was no evidence of tumour involvement in uterosacral ligaments in this study. This finding is noteworthy, as almost complete excision of these ligaments is performed in class III RAH. If confirmed by future studies, it may be more feasible to divide the uterosacral ligaments closer to the uterus.

This was a preliminary study that was conducted on a limited sample size. Future studies with larger sample sizes are needed to reach a final conclusion. Almost every gynaecological oncologist would agree that limiting the extent of cancer surgery should never impair oncological outcomes. With continuing research and the development of newer surgical techniques, patients' quality of life will be optimised accordingly.

Ethics Committee Approval: *Ethics committee approval was obtained from Başkent University Institutional Review Board (KA11/08).*

Informed Consent: *Informed consent was obtained from all study participants.*

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Relation between single serum progesterone assay and viability of the first trimester pregnancy

Tek serum progesteron tayini ile ilk trimesterde gebeliğin canlılığı arasındaki ilişki

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Abstract

Objective: This study was designed to detect the relation between serum progesterone and viability of pregnancy during the first trimester.

Material and Methods: Two hundred and sixty women during the first trimester of their pregnancies were hospitalised due to vaginal bleeding and/or abdominal pain and were included in this study. Criteria for inclusion in this study were: certain dates, foetus conceived spontaneously with no history of infertility and a positive serum pregnancy test. Blood samples were taken from women included in this study for serum progesterone assay; the patients were followed by ultrasound until the end of the first trimester for the viability of the pregnancy and the outcome of their pregnancy was recorded.

Results: By the end of the first trimester, women included in this study were classified into: viable pregnancy group (n=178; 68.5%) and non-viable pregnancy group (ended by miscarriage) (n=82; 31.5%). The mean serum progesterone of the studied population was significantly higher in the viable pregnancy group (46.5±7.4 ng/mL) compared to non-viable pregnancy group (9.9±4.8 ng/mL; $p<0.05$). The serum progesterone cut-off level of 10 ng/mL was 79.3% sensitive for diagnosing non-viable pregnancy and 93.3% specific for the diagnosis of viable pregnancy, while a cut-off level of 20 ng/mL was 95.1% sensitive for the diagnosis of non-viable pregnancy and 98.9% specific for diagnosing viable pregnancy.

Conclusion: Serum progesterone is a reliable marker for early pregnancy failure and a single assay of its serum level can differentiate between viable and non-viable pregnancies.

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Key words: First trimester, pregnancy, serum progesterone, single, viability.

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Özet

Amaç: Bu çalışma serum progesteron ile ilk trimesterde gebeliğin canlılığı arasındaki ilişkiyi saptamak için tasarlandı.

Gereç ve Yöntemler: İki yüz altmış kadın gebeliklerinin ilk trimesterinde vajinal kanama ve/veya abdominal ağrı nedeniyle hastaneye yatırıldı ve bu çalışmaya dahil edildi. Bu çalışma için dahil etme kriterleri şunlardı: kesin tarihler, infertilite öyküsü olmaksızın spontan oluşmuş fetüs ve pozitif serum gebelik testi. Bu çalışmaya dahil edilen kadınlardan serum progesteron tayini için kan örnekleri alındı; gebeliğin canlılığı açısından ilk trimesterin sonuna kadar hastalar ultrason ile takip edildi ve gebeliğin akibeti kaydedildi.

Bulgular: İlk trimesterin sonunda çalışmaya dahil edilen kadınlar şu şekilde sınıflandırıldı: canlı gebelik grubu (n=178; %68.5) ve canlı olmayan gebelik grubu (düşük ile sonlanan) (n=82; %31.5). Çalışılan popülasyonda ortalama serum progesteronu canlı olmayan gebelik grubuna kıyasla (9.9±4.8 ng/mL) canlı gebelik grubunda anlamlı şekilde daha yüksekti (46.5±7.4 ng/mL; $p<0.05$). Serum progesteron için 10 ng/mL'lik kesim (cut off) değeri canlı olmayan gebeliği teşhis etmede %79.3 sensitif ve canlı gebeliği teşhis etmede %93.3 spesifik iken 20 ng/mL'lik bir kesim değeri canlı olmayan gebeliği teşhis etmede %95.1 sensitif ve canlı gebeliği teşhis etmede %98.9 spesifikti.

Sonuç: Serum progesteronu erken gebelik kaybı açısından güvenilir bir belirteçtir ve serum seviyesinin tek bir analizi canlı ve canlı olmayan gebelikleri ayırt edebilir.

(J Turkish-German Gynecol Assoc 2013; 14: 68-71)

Anahtar kelimeler: İlk trimester, gebelik, serum progesteron, tek, canlılık.

Geliş Tarihi: 06 Kasım 2012 **Kabul Tarihi:** 12 Aralık 2012

Introduction

Ultrasound scanning is probably the best single diagnostic and prognostic test available for diagnosing early pregnancy failure. However, there were certain conditions where both sonographic and clinical findings were indeterminate or inconclusive (1).

Progesterone is a C-21 steroid hormone secreted by granulosa cells of the ovary. This hormone is important for promoting endometrial decidualisation to prepare the uterus for implantation of the blastocyst and to maintain the pregnancy (2). The physiological functions of progesterone include inhibition of smooth muscle contractility and inhibition of immune responses, like those involved in graft rejection (2).

Recent studies have suggested that serum progesterone measured in early pregnancy is the most powerful single predictor of pregnancy outcome in natural conceptions (1). Few

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studies have attempted to use serum progesterone assays to predict the outcome of pregnancy in *in-vitro* fertilisation (IVF)/ intra-cytoplasmic injection (ICSI) or in natural pregnancies, but none have produced convincing conclusions (3). It is essential to study women after natural conception without exogenous progesterone support, when the relation between serum progesterone and viability of the first trimester pregnancy was evaluated (4, 5). Therefore, this prospective study was designed to detect the relation between serum progesterone and viability of the pregnancy during the first trimester.

Material and Methods

This study was carried out over 3 years from February 2009 to February 2012. Two hundred and sixty (260) women were hospitalised due to vaginal bleeding and/or abdominal pain during the first trimester of their pregnancy and were included in this prospective study after providing informed consent, following approval of the study protocol by institute ethical committee of both hospitals (Ahmadi and Al-Rashid Hospitals).

Data were collected from women included in this study by direct questionnaire to identify age, parity, gestational age (calculated from the 1st day of last menstrual period (LMP)) and past history of early pregnancy miscarriages. Women included in the study were certain of dates, had conceived spontaneously with no history of infertility and had a positive serum pregnancy test.

Two mL blood samples were taken from women included in this study for serum progesterone assay; the samples were collected without anticoagulant in dry tubes. Serum was separated by centrifugation and stored at 2-8°C until hormonal assay was performed. The assay principle combines an enzyme immunoassay competition method with final fluorescent detection. Women included in the study were examined by ultrasound for viability of the pregnancy and accordingly the results were classified into viable and non-viable pregnancies. Those with inconclusive sonographic findings were re-examined by ultrasound after two weeks and were reclassified into viable and non-viable pregnancies according to the findings (anembryonic or missed miscarriage). Women included in this study were followed by ultrasound for the viability of the pregnancy until the end of first trimester and the outcome of their pregnancy was recorded, while women with exogenous progesterone support or multiple pregnancies or suspected ectopic pregnancy or hydatidiform mole were excluded from this study.

The ultrasound was done by an expert sonographer, who was blinded to the patients' data, using Philips HD9 (Philips HealthCare International, Amsterdam, Netherlands) with a 2D convex probe (4-9 MHz). Data were collected and statistically analysed to detect the relationship between serum progesterone level and viability of the pregnancy during first trimester.

Using data of previous studies (6, 7), setting the type-1 error (α) at 0.05, the power (1-β) at 0.8 and assuming a 5% dropout rate, the number of participants needed to produce a statistically acceptable figure was more than two hundred women. Data were collected, tabulated then statistically analysed using the Statistical Package for Social Sciences (SPSS) computer software version 15. Numerical variables were presented as mean and standard deviation (±SD), while categorical variables were presented as number and percentage. Chi-square test (X^2) was used for comparison between groups as regard qualitative variables. A difference with a p value <0.05 was considered statistically significant. Sensitivity is the proportional detection of individuals with the disease of interest in the population; specificity is the proportional detection of individuals without the disease of interest in the population.

Results

Two hundred and sixty women were hospitalised due to vaginal bleeding and/or abdominal pain during first trimester of their pregnancy and were included in this study. The mean age of the studied population was 32.7±5.1 years (ranged from 18-38 years), their mean parity was 4.2±5.7 (ranged from 0-9) and the mean gestational age at progesterone assay was 9.7±0.5 weeks (ranged from 7-11 weeks) (Table 1).

By the end of the first trimester, women included in this study were classified according to the viability of their pregnancies into the viable pregnancy group (n=178; 68.5%) and the non-viable pregnancy group (ended by miscarriages) (n=82; 31.5%); data are shown in Table 2.

The mean serum progesterone was significantly higher in viable pregnancy group (46.5±7.4 ng/mL; range 18.7-86.3 ng/mL), compared with non-viable pregnancy group (9.9±4.8 ng/mL; range 1.67-26.2 ng/mL) (Chi-square test, $p<0.05$); results are shown in Table 3.

The relations between serum progesterone and maternal age or gestational age of the studied populations were statistically insignificant; also, the relation between serum progesterone and past history of early miscarriage was statistically insignificant (Chi-square test; $p>0.05$); see Table 4.

In this study, 6.7% of viable pregnancies had a serum progesterone level <10 ng/mL, while 20.7% of the non-viable pregnancies had a serum progesterone level >10 ng/mL; the serum progesterone at a cut-off level of 10 ng/mL was 79.3% sensitive for the diagnosis of non-viable pregnancy and was 93.3% specific for diagnosing viable pregnancy. Also, in this study, 1.1% of viable pregnancies had a serum progesterone level <20 ng/mL, while

Table 1. The characteristics of the studied population

Variables	Mean±SD	Range
Age (Year)	32.7±5.1	18-38
Parity	4.2±5.7	0-9
Gestational age at progesterone assay (Weeks)	9.7±0.5	7-11

Table 2. Classification of the studied population according to the viability of the pregnancy

Ultrasound Findings	Number	Percentage
Viable pregnancy group	178	68.5%
Non-viable pregnancy group	82	31.5%
Missed abortion	53	20.4%
Anembryonic (blighted ovum)	29	11.1%
Total number of cases	260	100%

4.8% of non-viable pregnancies had a serum progesterone level >20 ng/mL; the serum progesterone at a cut-off level of 20 ng/mL was 95.1% sensitive for diagnosing non-viable pregnancy and 98.9% specific for the diagnosis of viable pregnancy (see Table 5).

Discussion

Recent studies suggest that serum progesterone measured in early pregnancy is the most powerful single predictor of pregnancy outcome in natural conceptions (1, 5, 6). Therefore, this prospective study was designed to detect the relation between serum progesterone and viability of the pregnancy during the first trimester.

Progesterone level and daily change in human chorionic gonadotropin (β-hCG) were determined in the serum of 307 patients with suspected ectopic pregnancy by Hahlin et al. (7), who found that 99% of the viable intrauterine pregnancies had serum progesterone more than 30 nmol/L (9.42 ng/mL; 1 nmol/1 = 0.314 ng/mL), whereas 75% of the ectopic pregnancies and 81% of the spontaneous abortions had serum progesterone less than 30 nmol/L (9.42 ng/mL). Also, serum samples were taken to assess progesterone, inhibin A, hCG, and urine β-core hCG from 220 women who presented in the first trimester of pregnancy with complaints of pain, cramping, bleeding or spotting by Phipps and colleagues, in order to evaluate whether those biomarkers could predict viable and non-viable outcomes in pregnancy; they concluded that serum progesterone was the most specific single biomarker for distinguishing viable from non-viable pregnancies (6).

However, Lijun et al. (8) concluded that serum progesterone combined with β-hCG measurements, with a diagnostic accuracy of 85.7%, had the best prognostic reliability for predicting the outcome of threatened miscarriage compared to serum progesterone alone or β-hCG alone. Daily et al. (9) found that the mean serum progesterone was significantly higher for viable pregnancies (22.1 ng/mL) compared to non-viable pregnancies (10.1 ng/mL) and they concluded that a serum progesterone assay alone is predictive of pregnancy outcome, especially during the first 8 weeks of gestation. Also, Al Jufairi (5) found that serum progesterone level was significantly higher in patients with viable pregnancies (20.48±6.066 ng/mL) compared with patients with non-viable pregnancies ended by spontaneous abortion (7.78±2.06 ng/mL); this author concluded that serum progesterone alone is a reliable marker for the prediction of early pregnancy failure.

The relations between serum progesterone and maternal age or gestational age of the studied population were statistically insignificant; also the relation between serum progesterone and past history of early miscarriage was statistically insignificant.

In this study, 6.7% of viable pregnancies had serum progesterone level <10 ng/mL, while 20.7% of non-viable pregnancies had serum progesterone level >10 ng/mL; the serum progesterone at a cut-off level of 10 ng/mL was 79.3% sensitive for the diagnosis of non-viable pregnancy and was 93.3% specific for diagnosing viable pregnancy. Also in this study, 1.1% of viable pregnancies had a serum progesterone level <20 ng/mL, while 4.8% of non-viable pregnancies had a serum progesterone level >20 ng/mL; serum progesterone at a cut-off level of 20 ng/mL was 95.1% sensitive for diagnosing non-viable pregnancies and was 98.9% specific for the diagnosis of viable pregnancy.

Ninety-five pregnant women of 13 weeks gestation or less were recruited as a study group and fourteen normal pregnant women were recruited as controls, to determine the role of serum progesterone as a marker of early pregnancy failure after single assay by Hanita et al. (2). They found that the serum progesterone levels were significantly lower in women with non-viable pregnancies compared with women with viable pregnancies (10.7 ng/mL vs. 45.9 ng/mL, respectively). Hanita et al. (2) concluded that serum progesterone can be used as a marker for early pregnancy failure

Table 3. The relation between serum progesterone and viability of the pregnancy

Pregnancy outcome	Number (%)	Serum Progesterone (ng/mL)	Test used p value (significance)
Viable Pregnancy group	178 (68.5%)	46.5±7.4 (18.7-86.3)	Chi-square (X^2) p<0.05 = 0.036 (significant)
Non-viable Pregnancy group	82 (31.5%)	9.9±4.8 (1.67-26.2)	

Table 4. The relation between serum progesterone and maternal age, gestational age or past history of early miscarriage

Variables	Number (%)	Serum Progesterone (ng/mL) Mean±SD	Test used p value (Significance)
Maternal age			Chi-square (X^2)
> 35 years old	142	24.62±8.2	p>0.05 = 0.76
< 35 years old	118	18.52±6.8	(Non-significant)
Past history of early miscarriage			Chi-square (X^2)
Positive	48	12.26±2.3	p>0.05=0.07
Negative	212	27.81±5.7	(Non-significant)
Gestational age			Chi-square (X^2)
> 10 weeks gestation	77	16.27±4.7	p>0.05 = 0.27
< 10 weeks gestation	183	26.45±3.9	(Non-significant)

Table 5. Relations between serum progesterone cut off levels and viability of the pregnancy

Variables	Viable Pregnancy group (Total number=178)	Non-viable Pregnancy group (Total number=82)
Serum Progesterone at cut off level 10 ng/mL		
Number of cases with serum progesterone <10 ng/mL (%)	12 (6.7%)	65 (79.3%=Sensitivity)
Number of cases with serum progesterone >10 ng/mL (%)	166 (93.3%=Specificity)	17 (20.7%)
Serum Progesterone at cut off level 20 ng/mL		
Number of cases with serum progesterone <20 ng/mL (%)	2 (1.1 %)	78 (95.1%=Sensitivity)
Number of cases with serum progesterone >20 ng/mL (%)	176 (98.9%=Specificity)	4 (4.8%)

and at a cut-off value of 32.7ng/mL serum progesterone had 90% sensitivity with 75% negative predictive value (NPV) and 92% specificity with 97% positive predictive value (PPV) to diagnose early pregnancy failure.

Four hundred and eighty-nine women presenting with singleton pregnancy, vaginal bleeding and/or abdominal pain in the first 18 weeks of pregnancy were included in a prospective comparative study by Al-Sebai et al. (10), to assess the role of a single maternal serum progesterone measurement in the immediate diagnosis of early pregnancy failure and in the long-term prognosis of foetal viability. They found that serum progesterone levels were significantly lower in the non-continuing and tubal pregnancy groups compared to threatened-continuing groups and a cut-off level of 45 nmol/L (14.13 ng/mL) was found to differentiate between the viable pregnancies and the abnormal (non-continuing) pregnancies with 87.6% sensitivity and 87.5% specificity. Al-Sebai et al. (10) concluded that a single serum progesterone measurement taken in early pregnancy is valuable in the immediate diagnosis of early pregnancy failure and the long-term prognosis of viability.

Also, a prospective study was conducted by Ioannidis and colleagues to investigate the relation between early (14 days after oocyte recovery) serum progesterone assay and pregnancy outcome in women undergoing in-vitro fertilization (IVF)/intracytoplasmic injection (ICSI) and receiving rectal progesterone supplements. They found that the single progesterone assay on day 14 post-oocyte retrieval was significantly higher in women with on-going pregnancies compared to women with an abnormal pregnancy. Ioannidis et al. (11) concluded that a single serum progesterone measurement could be a useful indicator of pregnancy outcome in women undergoing IVF/ICSI treatment. Also, the result of this study suggests that serum progesterone is a reliable marker for early pregnancy failure and that a single assay of its serum level can differentiate between viable and non-viable pregnancies. Future trials and large population studies are needed to support our findings and to establish the cut-off values of serum progesterone to differentiate between viable and non-viable pregnancies.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committees of both Ahmadi Hospital (Kuwait Oil Company) and Al-Rashid Hospital.*

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Does previous abdominal surgery effect the feasibility of total laparoscopic hysterectomy?

Geçirilmiş abdominal cerrahi total laparoskopik histerektominin uygulanabilirliğini etkilemekte midir?

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Abstract

Objective: The primary aim of this study is to evaluate the effects of previous abdominal surgery on the feasibility of performing and the safety of total laparoscopic hysterectomy (TLH).

Material and Methods: In this retrospective study, we analysed 62 laparoscopic hysterectomies which were performed at our institute between February 2011 and January 2013. We chose to perform laparoscopic surgery for all patients, including those who had previously undergone abdominal surgery. The patients were classified into two groups: Group 1 included patients with a history of abdominal surgery (n=24) and Group 2 included patients without a history of abdominal surgery (n=38).

Results: The operating period was compared in both groups: 184.43±51.0 min. for Group 1 and 195.41±64.1 min. for Group 2 (p=0.471). Postoperative hospital stay and blood loss was also compared. There was just 1 conversion from TLH to a laparotomy in both groups. None of the patients in Group 1 needed a blood transfusion, whereas 1 in Group 2 did.

Conclusion: We found that operation time, postoperative hospital stay, blood loss, rate of operative complications or conversion rate to open surgery between patients with and without a history of abdominal surgery were comparable. Therefore, it appears that a history of abdominal surgery does not adversely affect the safety of TLH.

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Key words: Laparoscopic hysterectomy, previous surgery, complications

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Özet

Amaç: Bu çalışmanın öncelikli amacı, daha önce geçirilmiş abdominal cerrahinin, total laparoskopik histerektomi (TLH) yapılabilirliği ve güvenliği üzerine etkisini değerlendirmektir.

Gereç ve Yöntemler: Bu retrospektif çalışmada, Şubat 2011 ve Ocak 2013 tarihleri arasında kurumumuzda gerçekleştirilen 62 TLH operasyonun kayıtları incelendi. Laparoskopik cerrahiyi, geçirilmiş abdominal cerrahisi olsa da tüm hastalarda düşündük. Hastalar iki gruba ayrıldı. Grup 1 geçirilmiş abdominal cerrahisi olanları, Grup 2 geçirilmiş abdominal cerrahisi olmayanları içerdi.

Bulgular: Operasyon süresi her iki grupta da karşılaştırılabilirdi (184.43±51.0 dk. Grup1 ve 195.41±64.1 dk. Grup 2; p=0.471). İki grup arasında intraoperatif komplikasyon açısından anlamlı fark bulunmadı. Operasyon sonrası hastanede kalış süresi ve kan kaybı da karşılaştırılabilirdi. Her iki grupta da sadece 1 vakada laparotomiye geçiş yapıldı. Grup 1'de hiçbir hastada kan transfüzyonu ihtiyacı olmazken, Grup 2'de 1 hastada oldu.

Sonuç: Çalışmamızda cerrahi geçirmiş ve geçirmemiş olan grup arasında, operasyon süresi, postoperatif kalış süresi, kan kaybı, operatif komplikasyon oranı veya açık cerrahiye geçiş oranı karşılaştırabilir bulundu. Sonuç olarak, abdominal cerrahi hikayesinin olması TLH nin güvenliğini olumsuz etkilememektedir.

(J Turkish-German Gynecol Assoc 2013; 14: 72-5)

Anahtar kelimeler: Laparoskopik histerektomi, geçirilmiş cerrahi, komplikasyonlar

Geliş Tarihi: 28 Şubat 2013 **Kabul Tarihi:** 07 Nisan 2013

Introduction

Hysterectomy is the most common major surgical procedure performed after Caesarean section in gynaecological surgical procedures. Traditionally, this procedure is performed through four routes: abdominal, vaginal, laparoscopic and, more recently, robot-assisted (1). Since its establishment in gynaecological surgery in the 1960s and 1970s, major improvements in visualisation technology and instrumentation in the mid-1980s have clearly endorsed laparoscopic techniques in gynaecology (2). Abdominal hysterectomy (AH), vaginal hysterectomy (VH) or total laparoscopic hysterectomy (TLH) can be chosen depending on the indication for surgery, the uterine size, the surgeon's experience, and patient characteristics and preference. There is still no consensus on the optimum method of hysterectomy (3).

The primary aim of our retrospective study was to evaluate the effects of previous abdominal surgery on the feasibility of performing and the safety of TLH in a single centre.

Material and Methods

We performed 62 laparoscopic hysterectomies at our institute between February 2011 and January 2013. We chose to perform laparoscopic surgery for all patients, including those who had previously undergone abdominal surgery.

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The patients were classified into two groups: Group 1 included patients with a history of abdominal surgery (n=24) and Group 2 included patients without a history of abdominal surgery (n=38). The study was approved by the Medical Ethical Committee of the Namik Kemal University and all participants signed informed consent.

Hysterectomy was performed in patients with myoma uteri, pelvic organ prolapsus, cervical intraepithelial neoplasia, chronic pelvic pain (pelvic pain longer than 6 months duration), ovarian cysts, and abnormal uterine bleeding resistant to medical treatment. All hysterectomies were performed by 3 surgeons with experience in advanced laparoscopic surgery. Exclusion criteria for TLH were poor uterine mobility, diagnosis of malignancy, uterine diameter of more than 10 cm (on ultrasonography), previous pelvic or abdominal irradiation, and severe cardiopulmonary disease precluding protracted Trandelenburg position. In addition, if patients did not consent for the study, a conventional abdominal hysterectomy was performed. The history of each patient's abdominal surgery, age, parity, indication for hysterectomy, operation time, postoperative hospital stay, blood loss, transfusion, peri- and postoperative complications, and conversion to open surgery were assessed. The patients were admitted to the hospital 1 day before for routine preoperative bowel preparation. Intraoperative bleeding was evaluated by an anaesthesiologist by quantifying the amount of blood in the aspirator container. Operative time began at anaesthesia and finished after skin closure.

Patients undergoing TLH were placed in the Trandelenburg position. At the beginning of total laparoscopic hysterectomy, the uterine cavity was measured and a colpotomiser system (KOH Cup Vaginal Fornices Delineator; CooperSurgical, Shelton, Connecticut, USA) was placed on the cervix and connected to a uterine manipulator (RUMI system, CooperSurgical, Shelton, Connecticut, USA). This step of the operation required approximately 10 minutes for each patient. We then proceed to the abdominal portion of the procedure. The laparoscopic operation was accomplished using an intraumbilical 10-mm 0-degree videolaparoscope (Hopkins, Storz, Tuttlingen, Germany). TLH was performed through 4 punctures with trocars: 1 in the umbilicus (direct view technique with insufflation), 2 lateral to the umbilicus to the right and to the left, and 1 in the left upper quadrant (transillumination and direct view). This allows the surgeon to operate with two hands and the assistant to run the camera and assist with counter-traction. Different tissue sealing devices (Enseal, Ethicon Endosurgery, Inc, Cincinnati, OH, USA: Ligasure, Covedien, Valleylab, Colorado, USA) have been reported for use in total laparoscopic hysterectomy. The dissection of adhesions is performed either sharply or with monopolar or bipolar energy sources. The totally amputated uterus was removed transvaginally; when the uterus was large, it was morcellated transvaginally. The vaginal vault was closed transabdominally; for suturing the vaginal cuff, we typically use traditional laparoscopic needle holders with number: 0 polyglactin (Vicryl) suture.

The statistical analysis was performed using Statistical Package Social Sciences for Windows software (Version 16.0 SPSS, Chicago, IL, USA). Data are presented as mean and SD or percentage. The Kolmogorov-Smirnov test was used to identify whether the variables were normally distributed. The differences between groups were assessed by using unpaired t-tests for parametric data and Mann-Whitney U-test for nonparametric data. Differences between groups were considered statistically significant at $p<0.05$.

Results

Patient characteristics such as body mass index (BMI) (29.36±5.5 vs. 29.59±5.0 kg/m^2; p=0.886) and age (50.03±7.21 vs. 50.54±6.0 years; p=0.754) did not differ between the two groups. The operating time was comparable between the two groups: 184.43±51.0 min for Group 1 and 195.41±64.1 min for Group 2 (p=0.471). Postoperative hospital stay (3.26±1.25 vs. 3.35±1.0 days; p=0.774) and blood loss (296.15±121.59 vs. 297.30±44.0 ml; p=0.958) were also found to be comparable (Table 1). Indications for hysterectomy and types of previous surgery are shown in Tables 2 and 3, respectively. Out of 24 patients, 1 had 3 abdominal surgeries and 2 had undergone 2 abdominal surgeries before. There was just 1 conversion from TLH to a laparotomy in both groups. None of the patients in Group 1 needed a blood transfusion, whereas 1 in Group 2 did; this patient received both intraoperative and postoperative transfusions. Regarding the intraoperative complications other than excessive bleeding, 1 case of ureteral trauma was seen in Group 2.

Discussion

Hysterectomy is the most frequent gynaecologic operation and TLH (61%) is the preferred approach for hysterectomy in our hospital. In our retrospective study, we compared the operative outcomes of TLH in the selected patient group according to their history of previous abdominal surgery at the single centre.

Table 1. General characteristics and clinical evaluation of patients

	Group 1 (n=24)	Group 2 (n=38)	p values
Age (years)	50.03±7.21	50.54±6.0	0.754
BMI (kg/m^2)	29.36±5.5	29.59±5.0	0.886
Estimated blood loss (mL)	296.15±121	297.30±44.0	0.958
Operating time (min)	184.43±51.0	195.41±64.1	0.471
Hospitalisation (d)	3.26±1.25	3.35±1.0	0.774
Data presented as mean±SD. A p value 0.05 is considered statistically significant			

Table 2. Indication for hysterectomy

	Group 1 (n=24)	Group 2 (n=38)
Myoma uteri	16	29
Pelvic organ prolapsus	1	4
Cervical intraepithelial neoplasia	-	1
Abnormal uterine bleeding	5	-
Chronic pelvic pain	-	2
Ovarian cyst	2	2

Table 3. Types of previous surgery

	n	%
Appendectomy	9	32.1
Laparoscopic cholecystectomy	3	10.7
Tubal ligation	4	14.2
Adnexal surgery	3	
Ovarian cyst	2	7.1
Ectopic pregnancy	1	3.5
Caesarean section*	6	21.4
Myomectomy	1	3.5
Others	2	
Colon operation	1	3.5
Intestinal perforation	1	3.5
*patient may have had more than one surgery		

The major concerns in performing laparoscopic surgery for patients with a history of abdominal surgery include the potential risk for access-related injury and the necessity for adhesiolysis and its attendant complications. In the present study, none of the patients showed perioperative complications resulting from trocar insertion. The access-related visceral injury incidence rate has been reported as 0.3-0.03% (4-6). A history of abdominal surgery increases the risk of adhesions between the abdominal wall and viscera (7). The potential for bowel injury during trocar placement or difficulty in visualisation of the structures has dissuaded some surgeons from performing a laparoscopic procedure in patients with a history of abdominal surgery. Some surgeons have reported the use of an open technique because of the safety afforded in patients with a history of abdominal surgery (8, 9).

In a previous study, it was reported that 59% of patients with a midline vertical incision had anterior wall adhesions. Twenty-eight per cent with a suprapubic transverse incision had anterior wall adhesions. Ninety-six per cent of adhesions involved the omentum and 29% included the bowel. According to these data, they concluded that prior laparotomy, whether through a midline vertical or suprapubic transverse incision, significantly increased the frequency of anterior abdominal wall adhesions, and that these adhesions may complicate the placement of the laparoscopic cannula through the umbilicus (7).

In the study conducted by Yada-Hashimoto et al. (10), the complication rate was similar in both groups (6.8 and 5.4%, respectively), and no bladder, bowel, ureteral or vascular injuries were encountered. However, in our study, the complication rate was higher in Group 1 (4.1% and 0%, respectively); no bowel or vascular injuries were encountered, except 1 cases of ureteral injury in Group 1 which was detected on the third day by ureteroscopic examination after surgery and ureteroneocystostomy was conducted via the abdomen. At the first month of follow-up, the patients were free of symptoms. The complication rate for TLH has gradually decreased with increased surgical experience at our institute; thus, less experienced gynaecologic surgeons may experience higher complications when attempting TLH. The reason for this difference in complication rate may be due to the small number of cases reported here which is not fully representative.

Again, in the study conducted by Yada-Hashimoto et al. (10), no statistical difference was reported in the TLH success rates in patients with and without a history of abdominal surgery. In that study, three patients with (6.8%) and two patients without (1.5%) a history of abdominal surgery converted to laparotomy. The total conversion rate of 2.9% in the that study is comparable to the rates reported in other TLH series (0.6-7%) (2, 9, 10, 11-13). Our results are consistent with these observations, as there was just one conversion from TLH to a laparotomy in both groups. In contrast to these findings, Sokol et al. (14) reported that pelvic adhesions and previous laparotomy were correlated with an increased risk of conversion to open surgery during gynaecologic laparoscopy. Leonard et al. (15) reported that uterine width, lateral myoma and a history of abdominopelvic surgery were significantly associated with laparoconversion in TLH patients.

In our study, we found that operation time, postoperative hospital stay, blood loss, rate of operative complications or conversion rate to open surgery between patients with and without a history of abdominal surgery were comparable. Therefore, it appears that a history of abdominal surgery does not adversely affect the safety of TLH.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Medical Ethical Committee of the Namik Kemal University (Approval number: 2013.42.04.02)*

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Lipoic acid decreases peritoneal adhesion formation in a rat uterine scar model

Lipoik asitin deneysel sıçan modelinde peritoneal adezyon oluşumunu azaltıcı etkisi

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Abstract

Objective: To investigate the effects of lipoic acid in the prevention of postoperative pelvic adhesions by a visual scoring system and immunohistochemistry in a rat uterine horn model with full thickness injury.

Material and Methods: Twenty-eight female Wistar albino rats were randomised into four groups: uterine trauma control, 15 days and 30 days, and uterine trauma + lipoic acid, 15 days and 30 days. A full thickness defect was established by incising a segment of approximately 1.0 cm in length from each uterine horn, leaving the mesometrium intact. Extension and severity of the adhesions in each group were scored by a visual scoring system and evaluated immunohistochemically.

Results: Adhesion scores were 2.00±0.81, 2.14±0.69 0.71±0.75, and 0.85±0.69 for extent and 2.28±0.48, 2.14±0.69, 0.85±0.69, and 1.14±0.69 for severity in Groups 1, 2, 3 and 4, respectively. Adhesion extent and severity were significantly less for groups treated by lipoic acid but no difference was observed between long and short administration. Both Vitronectin and u-PAR staining were significantly increased in treatment groups when compared to the control group.

Conclusion: Lipoic acid was found to be effective in reducing postoperative adhesion formation in a rat model.

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Key words: Lipoic acid, adhesion, rat, uterine scar, full thickness uterine injury

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Özet

Amaç: Tam kat uterus hasarı yapılmış deneysel sıçan modelinde lipoik asitin postoperatif pelvik adezyonlar üzerindeki etkisinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Yirmi sekiz dişi Wistar albino sıçan dört gruba randomize edildi. Uterin hasar grubu kontrol, 15 gün ve 30 gün, uterin hasar + lipoik asit grubu 15 ve 30 gün. Tam kat uterin hasar her bir uterin boynuzdan 1.0 cm uzunlukta, mezometriyumu salim bırakarak insizyon yapılarak ve sütüre edilerek oluşturuldu. Adezyonların yaygınlığı ve şiddeti görsel skorlama sistemi ile ve immunohistokimyasal olarak yapıldı.

Bulgular: Adezyon skorları grup 1, 2, 3 ve 4 için sırasıyla yaygınlık: 2.00±0.81, 2.14±0,69 0.71±0.75, 0.85±0.69 ve şiddet: 2.28±0.48, 2.14±0.69, 0.85±0.69,1.14±0.69 olarak belirlendi. Adezyon yaygınlık ve şiddeti lipoik asit ile tedavi edilen grupta anlamlı olarak daha azdı ancak kısa veya uzun süreli kullanım arasında fark yoktu. Hem Vitronectin hem u-Par boyanması tedavi grubunda kontrole göre anlamlı olarak artmıştı.

Sonuç: Lipoik asit deneysel sıçan modelinde postoperatif adezyon oluşumunu önlemede etkili bulundu.

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Anahtar kelimeler: Lipoik asit, adezyon, sıçan, uterus hasarı, tam kat uterin hasar

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Introduction

The process of incision, cauterisation and suturing during surgery inevitably results in tissue healing and postoperative peritoneal adhesions, which may lead to female infertility, chronic abdominal pain or bowel obstruction (1, 2). Safe surgical entry into the abdomen is difficult in subsequent interventions and the risk of intestinal injury, haemorrhage and inadequate site exposure is increased. Minimalising tissue trauma, the avoidance of foreign materials and prophylaxis for infection are common measures against adhesion formation. There are numerous studies on developing agents such as barrier materials, hormones and their agonist/antagonists, hyaluronic acid, fibrinolytic agents, non-steroidal anti-inflammatory drugs and antioxidants to prevent postoperative adhesion formation (3-7).

Women with leiomyoma uteri who wish to preserve their uterus undergo myomectomy operations; approximately 65,000 myomectomies are performed annually in the USA (8). Haemostasis may fail during myomectomy, resulting in postoperative adhesions impairing fertility (9). The caesarean rate for the USA is reported as 30.3%; this many uterine cavities are exposed during surgery and are then sutured (10). These two frequent operations contribute to the risk of

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peritoneal adhesions. Adhesion formation following uterine scarring in animal experiments has not been reported to date. In this study, we used a rat model resembling myomectomy or caesarean section with uterine scarring and suturing and tested the effect of Lipoic Acid (LA) on postoperative peritoneal adhesion formation.

Free radicals, namely superoxides, peroxides and hydroxyl radicals, are mediators of inflammation inducing adhesions by cellular membrane damage. Antioxidants such as methylene blue, vitamin E and N-acetyl cysteine have been reported to decrease development of peritoneal adhesions (7, 11, 12). There are few studies to our knowledge investigating the effect of LA in the prevention of adhesion formation. In this study, we aim to evaluate the effects of LA in the prevention of postoperative pelvic adhesions. To rule out subjective evaluation we have used both a visual scoring system and immunohistochemically we have used the wound healing markers, urokinase plasminogen activator (u-PAR) and vitronectin in a rat uterine horn model with full thickness injury of the myometrium.

Material and Methods

Twenty-eight female, non-pregnant Wistar albino rats were used; all experiments were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of Dokuz Eylül University Faculty of Medicine. The rats weighed approximately 200-250 g, were housed three animals to a cage under standard laboratory conditions with a day cycle of 14 hours light and had free access to food and water. The rats were randomly assigned to one of four study groups: Group 1, uterine scar group (15 days) (n=7); Group 2, uterine scar group (30 days) (n=7); Group 3, uterine scar+LA therapy (15 days) (n=7); and Group 4, uterine scar+LA (30 days) (n=7).

To standardise the hormonal status of the rats, menstrual cycle was determined by vaginal smear and the experiment was done on day of the dioestrus phase. Experimental design was modified the full-thickness injury model described in 2012 by Lin et al. (13) All rats were anaesthetised by intraperitoneal injection of ketamine and xylazine (35 mg/kg). The first incision was made in the abdominal wall of each rat under sterilised conditions. Then a full-thickness defect was created by incising a segment of approximately 1.0 cm in length from each uterine horn, leaving the mesometrium intact (Figure 1). The margins of the uterine defect were marked with a 4-0 nylon line. The abdominal incision was closed in two layers with a monofilament 3/0 polyglactin suture for the peritoneum and 2/0 polyglactin suture for the skin. The operation time did not exceed 15 minutes and all animals recovered without any complications or infections. All animals were treated with an intramuscular injection of penicillin (80.000 units/100 mg) for 3 days after the surgery.

Alpha lipoic acid (Sigma, St Louis, MO, USA) was prepared by mixing 100 mg/kg with sterile saline in a dark bottle and adding 1 M NaOH until the suspension dissolved. The pH was adjusted to 7.4 by adding 1 M HCl. Fresh LA solution was administered by oral gavage for either 15 or 30 days after uterine scarring.

After 15 or 30 days according to the study groups, the animals were anaesthetised, relaparatomy was performed, the extent and severity of intraabdominal adhesions were recorded and animals were sacrificed. All uterine horns of each rat in all study groups were evaluated separately (total 14 horns).

An author blinded to the medication status of the rats performed the visual assessment of adhesions. A published scoring system was used (14). The extent was evaluated as 0 for no adhesions, 1 for 25% of adhesions of the traumatised area, 2 for 50% of adhesions of the traumatised area and 3 for total involvement. The severity scores were 0 for no resistance to separation, 1 when minimal dissection was required and 3 for sharp dissection.

Uterine horns were fixed in neutral formalin fluid, dehydrated in graded series with ethanol and water, and embedded in paraffin. Serial sections 5 microns thick were collected on slides. For light microscope evaluation, haematoxylin eosin staining was performed. Streptavidin-biotin technique was used for immunostaining with u-PAR (rabbit anti-rat urokinase receptor IgG, 3920, American Diagnostica; 10 mg/mL concentration) and Vitronectin (ab45139, Abcam; 1/100 dilution). Following overnight incubation at 60°C, sections were dewaxed in xylene for 20 minutes. A decreasing series of ethanol was used for rehydration and then sections were washed in distilled water followed by phosphate-buffered saline (PBS) for 10 min each. Then, they were treated with trypsin (Cat No: 00-3008 Digest All 2A, Zymed, San Francisco, CA, USA) at 37°C for 15 min. To inhibit endogenous peroxidase activity, sections were delineated with a Dako pen (Dako, Glostrup, Denmark) and incubated in a solution of 3% H_2O_2 for 15 min. Sections were incubated with a blocking solution (Invitrogen, Histostain-Plus Broad Spectrum Cat No:

Figure 1. a, b. Macroscopic appearance of adhesion areas c. Haematoxylin-eosin evaluation of adhesion areas

85-9043) and primary antibodies for uPAR and Vitronectin for 18 hours. Then, the sections were incubated with biotinylated IgG (Invitrogen, Histostain-Plus Broad Spectrum Cat No: 85-9043) for 30 min, and then with streptavidin-peroxidase conjugate (Invitrogen, Histostain-Plus Broad Spectrum Cat No: 85-9043) for 30 min. Finally, they were incubated with a solution containing DAB (Catalogue no: 1718096, Roche) to visualise immunolabelling, and counterstained with Mayer's haematoxylin (HMM199, ScyTec, Logan, Utah, USA). They were then washed with distilled water three times and mounted with entellan. All sections were examined using a light microscope.
Positive stained cells among 100 cells in 5 fields randomly chosen in each horn were counted. A total of 500 cells were counted for each horn. Statistical analysis was done using SPSS 15.0 (SPSS Inc., Chicago, USA) and Kruskal-Wallis test was applied. Results were given as mean +/- standard deviation.

Results

The extent of adhesions was 2.00±0.81, 2.14±0.69 0.71±0.75, and 0.85±0.69 for Groups 1, 2, 3 and 4, respectively. Adhesion extent was significantly less for lipoic acid groups. When comparing 15 days and 30 days of LA administration, there was no difference. The severity of adhesions was 2.28±0.48, 2.14±0.69, 0.85±0.69 and 1.14±0.69 for Groups 1, 2, 3 and 4, respectively. Severity of adhesions was significantly less in the LA groups, but no difference was observed between long and short administration (Table 1).
Both Vitronectin and u-PAR staining were significantly increased in LA groups when compared to the scar group. There was no significant difference between short and long LA application groups (Table 2, Figure 2).

Discussion

Postoperative pelvic adhesions may lead to complications such as extended operation time, additional blood loss and visceral damage (7) in cases of relaparatomy. Caesarean sections are the most frequently performed obstetrical operations worldwide and recurrent caesareans are difficult due to intraabdominal adhesions. Similarly, after a myomectomy there is a risk of peritoneal adhesion formation. In this study, we demonstrated that when applied orally, both short and long duration treatments with lipoic acid were effective in preventing pelvic adhesions following surgical trauma in rats. Tissue remodelling markers were used to verify the results.
The incision site of the myometrium and locations of subsequent suturing are areas where wound healing takes place. Healing is a result of proliferation and regeneration of the mesothelial cell layer and fibrinolysis producing a peritoneal scar (15). Adhesions are primarily the result of this scar. An increase in oxidative stress and the formation of reactive oxygen species (ROS) play an important role in the pathophysiology of adhesion formation. Antioxidants such as methylene blue, vitamin E and N-acetyl cysteine have been reported to decrease development of peritoneal adhesions (7, 11, 12). Alpha LA and its metabolites are antioxidant and, when in contact with free radicals, oxidation takes place (16).
There are several experimental models for producing peritoneal adhesions in laboratory animals: the damaged uterine horn model by electrocautary or scraping, caecal abrasion, peritonitis model and the bowel anastomosis model (4, 17-20). The traumatisation of the uterine horn is widely used to mimic abdominal surgery; however, in the most frequent gynaecology

Table 1. Extent and severity of adhesion

Group	Uterine horn number (mean±SD)	Adhesion extent (mean±SD)	Adhesion severity (mean±SD)
Uterine Scar Group (15d)	14	2.00±0.81	2.28±0.48
Uterine Scar Group (30d)	14	2.14±0.69	2.14±0.69
Uterine Scar Group+LA therapy (15d)	14	0.71±0.75*	0.85±0.69*
Uterine Scar Group+LA therapy (30d)	14	0.85±0.69**	1.14±0.69**

Uterine Scar 15 d vs. Uterine Scar 30 d (p=0.72, p=0.70) for extent and severity; (*) Uterine Scar 15 d vs. Uterine Scar 15 d+LA (p=0.01, p=0.003) for extent and severity; (**) Uterine Scar 30 d vs. Uterine Scar 30 d+LA (p=0.009, p=0.024) for extent and severity, Uterine Scar 15 d+LA vs. Uterine Scar 30 d+LA (p=0.674, p=0.431) for extent and severity

Table 2. Immunohistochemical staining of Vitronectin and u-PAR of adhesion tissue

Group	Vitronectin (mean±SD)	u-PAR (mean±SD)
Uterine Scar Group (15d)	11.42±2.50*	13.7±2.05*
Uterine Scar Group (30d)	21.43±6.57	19.4±3.86
Uterine Scar Group+LA therapy (15d)	39.14±9.51**	40.7±8.42**
Uterine Scar Group+LA therapy (30d)	42.14±8.98***	43.5±6.72***

(*) Uterine Scar 15 d vs. Uterine Scar 30 d (p=0.001, p=0.011) for Vitronectin and u-PAR immunostaining; (**) Uterine Scar 15 d vs. Uterine Scar 15 d+LA (p=0.001, p=0.001) Vitronectin and u-PAR immunostaining; (***) Uterine Scar 30 d vs. Uterine Scar 30 d+LA (p=0.004, p=0.001) for Vitronectin and u-PAR immunostaining, Uterine Scar 15 d+LA vs. Uterine Scar 30 d+LA (p=0.902, p=0.456) for Vitronectin and u-PAR immunostaining

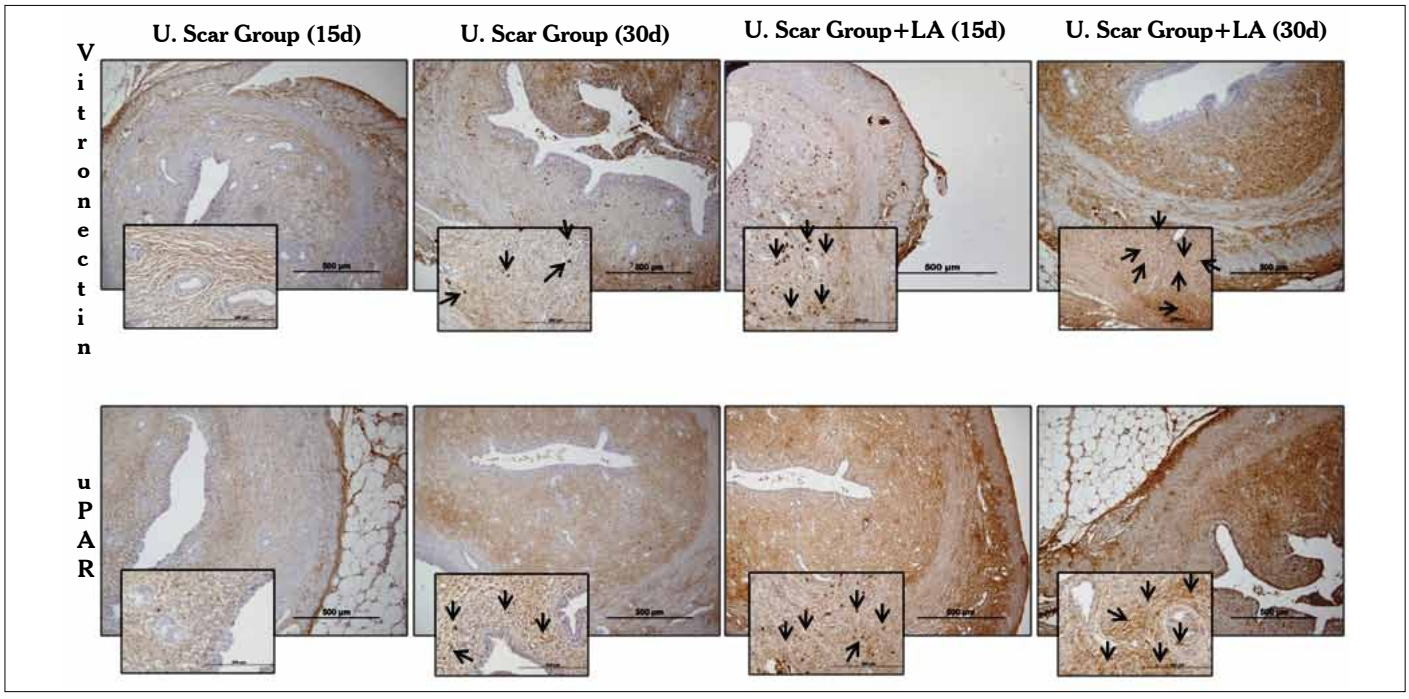

Figure 1. Staining of vitronectin and u-PAR in uterine scar groups of control and LA after 15 and 30 days (*arrows*)

and obstetrics operations there is a full thickness cut through the uterine wall. Therefore, we have chosen a new model to form adhesions. We cut the uterine horns using full layer thickness and then sutured the incision resembling a caesarean section or myomectomy where the uterine cavity is exposed.

Tissue remodelling and a normal healing process require plasminogen activators to form plasmin which will play a role in fibrinolysis. Insufficiency in fibrinolysis after surgery may lead to fibrin deposition causing adhesions. Urokinase plasmin activator binds to its receptor u-PAR which was used in the present study. Vitronectin on the other hand activates plasminogen activators and integrins and the balance of these molecules is important in regeneration (21, 22).

ROS are shown to be involved in adhesion formation after surgery. There is an increase of free radical activity of superoxide anions, xanthine oxidase and MDA (23, 24). The surgical area is a local hypoxic environment leading to an ischemia/reperfusion process resulting in a decrease of free radical scavenger levels. Restoration of these free radical scavengers have been shown to prevent adhesion formation in animal studies with induced intestinal ischemia (25). During peritoneal healing, oxidative stress increases and a positive correlation between the level of oxidative stress and the severity of adhesions has been demonstrated (26, 27). Postsurgical adhesion formation was reduced by the administration of antioxidants such as vitamin E, selenium or resveratrol in previous studies (5, 12, 28). Ozler et al. (29) have shown the existence of oxidative stress in a rat model with caecal trauma and a decrease after the application of lipoic acid. Even though the method of adhesion formation induction differs from our study, it supports our hypothesis that LA is effective in preventing postsurgical adhesions.

Lipoic acid is used in the therapy of diabetes, atherosclerosis, neurodegenerative processes, joint diseases or acquired immune deficiency syndrome (16, 30). It has a low redox potential and thus participates in reactions neutralising ROS, and reduces the oxidised forms of other antioxidants. Another advantage of LA is the fact that it is soluble in water and in fats (16). This is the first experimental study that combines a new method for adhesion formation, an antioxidant molecule (lipoic acid) and immunohistochemical methods to evaluate the results objectively.

Most studies about postoperative adhesion formation and prevention use visual evaluation methods and remain as subjective results. The authors have contributed to the literature on adhesion evaluation via an immunohistochemical method using u-PAR and vitronectin markers for objective evaluation (4). In the present study, this method is used again to confirm the anti-adhesion effect of lipoic acid administration after surgical intervention mimicking caesarean section or myomectomy. A distinctive increase in both tissue regeneration markers has been observed in animals treated with LA. The present results suggest a possible preventive effect of LA on postoperative adhesions after full thickness uterine trauma with minimal side effects and minimal cost.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Animal Care and Use Committee of Dokuz Eylül University Faculty of Medicine.*

Informed Consent: *N/A.*

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Melatonin treatment results in regression of endometriotic lesions in an ooferectomized rat endometriosis model

Melatonin tedavisi ooferektomize sıçanlar endometriozis modelinde endometriotik odaklarda gerilemeye yol açar

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Abstract

Objective: We aimed to determine the effects of melatonin treatment on endometrial implants in an oopherectomized rat endometriosis model.

Material and Methods: This study is a prospective, randomised, controlled experimental study. It was carried out at the Experimental Research Center of Yeditepe University (YUDETAM). Twenty-two, female, non-pregnant, nulligravid Spraque-Dawley albino rats were included in our study. Endometriosis was surgically induced in oopherectomized rats. Rats were randomised into two groups: control group and melatonin group. In the melatonin group, rats were treated with melatonin (20 mg/kg/day) for two weeks. After the operations were performed to assess the regression of the endometriotic lesions, melatonin treatment was stopped. At the end of the sixth week necropsies were performed to assess the rate of recurrence. The volume and histopathological scores of endometriotic foci were examined.

Results: Volumes of the endometriotic lesions significantly decreased in the melatonin group. Also, when the melatonin group was analysed within itself, endometriotic lesion volumes decreased and histopathological scores increased significantly.

Conclusion: Melatonin causes regression of the endometriotic lesions in rats and improvement in their histopathological scores.

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Key words: Endometriosis, oopherectomized rats, volume, histopathological score, melatonin

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Özet

Amaç: Ooferektomize sıçan endometriozis modelinde melatoninin endometriotik odaklar üzerine etkisini incelemeyi amaçladık

Gereç ve Yöntemler: Bu çalışma prospektif, randomize, kontrollü, deneysel bir çalışmadır. Çalışma Yeditepe Üniversitesi Deneysel Araştırma Merkezinde (YÜDETAM) yapıldı. Deneysel çalışmamıza 22 adet dişi, gebe olmayan, nulligravid Spraque-Dawley albino sıçan dahil edildi. Ooferektomize edilen sıçanlarda cerrahi olarak endometriozis oluşturuldu. Sıçanlar iki gruba ayrıldı: kontrol grubu ve melatonin grubu. Melatonin grubunda 2 hafta boyunca sıçanlara melatonin (20 mg/kg/gün) tedavisi verildi. Endometriotik regresyonu değerlendirmek için operasyonlar yapıldıktan sonra melatonin tedavisi sonlandırıldı. Rekürrens oranlarını değerlendirmek için altıncı haftanın sonunda nekropsiler yapıldı. Endometriotik odakların hacim ve histopatolojik skorları değerlendirildi.

Bulgular: Endometriotik lezyonların hacimleri melatonin grubunda anlamlı derecede azaldı. Ayrıca, melatonin grubu kendi içerisinde incelendiğinde endometriotik lezyonların hacimlerinin anlamlı derecede azaldı ve histopatolojik skorları anlamlı derecede arttı.

Sonuç: Melatonin sıçanlarda endometriotik odakların gerilemesine ve histopatolojik skorların iyileşmesine neden olur.

(J Turkish-German Gynecol Assoc 2013; 14: 81-6)

Anahtar kelimeler: Endometriozis, ooferektomize sıçan, hacim, histopatolojik skor, melatonin

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Introduction

Endometriosis is the presence of endometrial tissue with glands and stroma outside the uterus (1). It is a benign disease, but its clinical spectrum varies widely. It is associated with both pelvic pain and infertility and is prone to progression and recurrence.

Several pathogenic mechanisms including retrograde menstruation and implantation, coelomic metaplasia, direct transplantation, and vascular dissemination have been proposed

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in the aetiopathogenesis of endometriosis. However, no one mechanism explains all cases of endometriosis and each is thought to contribute, at least to some extent (2).
Sampson's theory of retrograde menstruation is considered the most commonly accepted theory for the development of the disease, at least in its form of peritoneal implants (3). Although retrograde menstruation occurs in at least 76-90% of women undergoing peritoneal dialysis and laparoscopy, the prevalence of endometriosis is much lower (6.2-8.2%) (4-7). These findings suggest that other factors must determine the susceptibility to developing endometriosis.
It was stated that the ability of endometrial implants to survive in ectopic locations may be due to an aberrant immune response (8). Oxidative stress also has been proposed as a potential factor in the pathophysiology of the disease (9). Inducers of oxidative stress may include erythrocytes, apoptotic endometrial cells, and undigested endometrial cells in the menstrual effluent (10).
Several studies have indicated that antioxidant defences may be altered in endometriosis, as suggested by the aberrant expression of endometrial antioxidant enzymes and lower levels of the antioxidant vitamin E in peritoneal fluid (11-14).
Endometriosis has the unique characteristics of invasion and requires the remodelling of the extracellular matrix (ECM) (15-17). Matrix metalloproteinases (MMPs) are a series of zinc-requiring proteolytic enzymes that are involved in the remodelling and degradation of the ECM. Derangement of MMP regulation is considered to be a critical factor in the development of pathological conditions like endometriosis (18, 19).
Recently, Collette et al. (20) reported that eutopic endometrium of women with endometriosis shows increased activity of MMP-9. Studies also demonstrated that MMP-3 expression is elevated in ectopic endometrial tissues of rats surgically induced to develop endometriosis (21, 22).
Matrix metalloproteinase activity can be modulated by oxidative stress (23, 24). Both MMP-2 and MMP-9 are activated by reactive oxygen species (ROS), and their expression seems to be regulated by oxidant stress (25). The role of ROS in increasing the growth and adhesion of endometrial cells in the peritoneal cavity during endometriosis has been documented (26, 27). Administration of antioxidant enzymes like superoxide dismutase and catalase has been shown to prevent intraperitoneal (i.p.) adhesions of endometriotic tissues in the peritoneal cavity of rabbits (28).
Melatonin (N-acetyl-5-methoxy-tryptamine) is the main pineal hormone synthesised from tryptophan, predominantly during the night (28). Melatonin is critical for the regulation of circadian and seasonal changes in various aspects of physiology and neuroendocrine function (29, 30).
Melatonin is a documented powerful free radical scavenger and a broad-spectrum antioxidant (31). Therefore, it may interfere with the oxidative stress seen in endometriosis.
It may also have an impact on the extracellular matrix remodelling seen in this disease, through the regulation of the zinc-requiring proteolytic enzymes MMPs, as there is indirect evidence that melatonin inhibits the production of adhesion molecules that promote the binding of leukocytes to endothelial cells (32-34). Therefore, melatonin may be an effective treatment modality in endometriosis.
In this study, we investigated the efficacy of melatonin in an oopherectomized rat endometriosis model with continuous high dose exogenous oestrogen administration.

Material and Methods

Twenty-two reproductive aged female non-pregnant, nulligravid Spraque-Dawley albino rats weighing 200-250 gr, which were bred at Yeditepe University Experimental Research Center (YUDETAM), were used in this study.
The rats were caged individually in a controlled environment. The room temperature was 21°C and humidity was 60%. Rats were fed ad libitum with 12 hour light/dark cycles.
After the induction of endometriosis according to experimental animal model, rats were randomised into two groups (control and 20 mg/kg/day Melatonin group). There were 10 rats in the melatonin group and 12 rats in the control group.
Rats in both groups were administered high dose oestrogen (50 μg/kg; twice weekly) until the end of the study (6 weeks). Melatonin was only given for two weeks to visualise melatonin-related endometriotic implant regression and recurrence.
Oophorectomy was performed to stabilise and standardise the oestrogen levels in rats throughout the study.
This study was approved by the Experimental Animals Ethics Committee of Yeditepe University. All experiments were performed in compliance with international guidelines on the ethical use of animals. Throughout the study period animals were controlled daily by a veterinary doctor and her assistant.

Surgical techniques

All rats had four operations. In the first operation after oophorectomy, endometriosis was induced by using homologous uterine horn autotransplantation. After two weeks of oestradiol treatment the second operations were performed to visualise endometriotic lesion formation. Then, rats were randomised using a randomisation table into the control and melatonin groups. Melatonin was administered only to the treatment group for two weeks. The third operations were performed for the assessments of the effects of melatonin on the endometriotic foci. After this inspection melatonin was stopped. Two weeks later (at the end of the sixth week) all of the rats were euthanised and the recurrence of endometriosis after melatonin effects was compared to the control group.

First operation: Endometriosis induction

All rats were anaesthetised with an intramuscular administration of 60 mg/kg ketamine hydrochloride (Ketalar; Eczacıbaşı İlaç Sanayi, İstanbul, Turkey) containing with 7 mg/kg xylazain hydrochloride (Rompun; Bayer İlac Sanayi, Istanbul, Turkey) as described previously (35-37). Endometriosis was induced surgically under anaesthesia as proposed by Vernon and Wilson, with modifications by Lebovic and Uygur et al. (38-40). After administration of general anaesthesia, the abdominal cavity was opened using a vertical incision. Ovaries and

uterine horns were visualised and all were removed by en block resection. Cauterisation was performed for coagulation, and uterine horns and ovaries were placed in phosphate-buffered saline at 37°C. Ovaries were separated from uterine horns, and parametrial fatty tissue was discarded. Cylindrical-shaped uterine horns were hold and cut longitudinally to expose the inner endometrial covered layer. Four pieces of grafts measuring 6x3x1 mm were made by division of the uterine horns. Two of these were implanted with 6/0 non-absorbable polypropylene suture on the peritoneum, where bifurcation of the abdominal wall vasculature was located in the right hypochondrial area. The others were implanted on the left site. During implantation, endometrial surfaces of grafts were attached to the peritoneum. The peritoneal cavity was kept moist with sterile saline solution throughout the surgery. The midline abdominal incision was closed in a continuous interlocking manner with 3-0 vicryl sutures. All rats were given 50 mg/kg/day cefazolin sodium (IE Ulagay İlaç Sanayi, İstanbul, Turkey) intramuscularly for 3 days after the operation. Also, oestrogen
(Oestradiol powder, ≥%98, Sigma-Aldrich) treatment was given to all rats (50 μg/kg) two times per week subcutaneously until the end of study.

Second operation: Assessment of the endometriotic foci
Two weeks after the first operation (endometriosis induction), the second was performed to assess the endometriotic lesions. Exogenous high dose oestrogen created a hyper-estrogenic state and resulted in well-defined endometriotic lesions. The second operations were performed using the aforementioned methodologies. Before endometriotic lesions were biopsied, all of the implants were measured by the same author (N.C.) in three dimensions (length - width - height in millimetres) using a ruler.
One of the four implants was removed for histopathological analysis using a randomisation table. After this operation, rats were randomised into two groups: control and melatonin treatment groups; 20 mg/kg/day melatonin treatment was administered using intramuscular and intraperitoneal routes in the treatment group. Melatonin was given for 2 weeks (until the third operation).

Third operation: The effects of melatonin
In the third operation, which was performed after melatonin treatment (at the end of 4th week), volumes of endometriotic foci were measured as previously described. One of the endometriotic lesions was randomly collected for histopathological examination. Melatonin treatment was stopped and oestrogen administration was continued.

Forth operation-necropsy: Evaluation of recurrence
The recurrence of endometriosis was evaluated after cessation of melatonin; during the last two weeks, rats were administered only oestradiol. All rats were euthanised under general anaesthesia and all measurements and endometriotic foci collections (all rest foci) were performed as described before.

Volume analysis
The spherical volume of each ectopic uterine tissue was calculated using the prolate ellipsoid formula: V (mm^3) = 0.52 x length x width x height (all in millimetres) (35, 40).

Histopathological analysis
Endometriotic biopsy samples were fixed in 10% neutral buffered formaldehyde solution. After dehydration, all pieces were embedded in paraffin. 3 μm thick sections were made with a microtome. Samples were stained with haematoxylin and eosin (HE), and were examined under a light microscope. The pathologist (FO) who assessed the samples was blinded to the treatment groups. Epithelial cells in the implants were evaluated as described by Keenan et al. (41). Based on this scoring system, lesions were given points as following:
Point 3: if epithelial surface layers were well preserved,
Point 2: if moderate leukocytic infiltration was seen,
Point 1: poor epithelial lining,
Point 0: if no epithelial lining was seen.

Statistical analysis
Statistical analysis was performed by using SPSS, version 11.5 (SPSS Inc., Chicago, IL, USA) for windows. Data were expressed as mean±standard error of mean. When these parameters were compared between the groups, Kruskal-Wallis Test with Mann-Whitney U test as a post-hoc test was performed. Friedman's Test with Wilcoxon as a post hoc test was used for the evaluation of lesion volumes and histopathological scores throughout the study in each group. $p<0.05$ was considered as statistically significant.

Results

We operated upon twenty-two rats and implanted 88 uterine graft tissues. Endometriotic foci were seen in each of the implanted grafts at the second operation. No graft failure was seen. Throughout the study there were no complications related to operations or toxic effects of melatonin. Some of the endometriotic foci were cystic in nature and the others were hyper-pigmented thickened lesions.

Comparison of the lesions between control and melatonin groups
During the second operation, which was performed 2 weeks after endometriosis induction, mean lesion volumes were (Volume A) 108.2 mm±17.5 (SEM) and 124.5 mm±14.8 (SEM), and histopathology scores were (Histology A) 1.7±0.1 (SEM) and 2.2±0.2 (SEM) in the melatonin and control groups, respectively (Table 1). There were no statistically significant differences in the lesion volumes or histopathology scores between the groups.
After this operation, 20 mg/kg/day melatonin treatment was administered using intramuscular and intraperitoneal routes in the treatment group. Melatonin was given for 2 weeks (until the third operation).
Two weeks after melatonin treatment the third operations were performed. Mean lesions volumes were (Volume B) 25.8 mm±3.6 (SEM) and 122.4 mm±23.1 (SEM) and histopathology scores were (Histology B) 2.2±0.2 (SEM) and 2.4±0.3 (SEM)

in the melatonin and control groups, respectively (Table 1). The endometriotic lesion volumes were significantly lower (p=0.001) in the melatonin group, while there were insignificant differences in histopathology scores between the groups.
During the necropsy operations, endometriotic lesion volumes were again significantly lower in the melatonin group (p=0.001), while there were insignificant differences in histopathology scores between the groups. Mean lesion volumes were (Volume C) 32.7 mm±6.0 (SEM) and 109.2 mm±12.8 (SEM) and histopathology scores were (Histology C) 2.7±0.2 (SEM) and 2.6±0.2 (SEM) in the melatonin and control groups, respectively (Table 1).

Comparison of the lesions within each group

a. 20 mg/kg/day melatonin group
Lesion volumes of this group were (Volume A, B, C); 108.2 mm±17.5 (SEM), 25.8 mm±3.6 (SEM) and 32.7 mm±6 (SEM) at the second, third and fourth operations, respectively. There were statistically significant decreases in lesion volumes of this group (p=0.001). "Volume B" values were lower than "Volume A" (p=0.03) and "Volume C" values were lower than "Volume A" (p=0.03). "Volume C" was insignificantly higher than "Volume B". Histopathology scores were (Histology A, B, C); 1.7±0.1 (SEM), 2.2±0.2 (SEM) and 2.7±0.2 (SEM) at the second, third and fourth operations, respectively. There were statistically significant increases in histopathology scores in this group (p=0.009). "Histology C" scores were higher than "Histology A" scores (p=0.02). "Histology B" scores were insignificantly higher than "Histology A" scores and "Histology C" scores were insignificantly higher than "Histology B" scores (Table 2).

b. Control group
Mean lesion volumes of this group were (Volume A, B, C); 124.5 mm±14.8 (SEM), 122.4 mm±23.1 (SEM) and 109.2 mm±12.8 (SEM); mean histopathology scores (Histology A, B, C) were 2.2±0.2 (SEM), 2.4±0.3 (SEM) and 2.6±0.2 (SEM) at the second, third and fourth operations, respectively (Table 2).
There were no statistically significant differences in lesion volumes (Volume A, B, C) and histopathology (Histology A, B, C) scores in the control group.

Table 1. Immunohistochemical staining of Vitronectin and u-PAR of adhesion tissue

	Melatonin Group Mean ± SEM		Control Group Mean ± SEM		p
2nd Operation					
Volume A	108.2	17.5	124.5	14.8	0.54
Histopathology A	1.7	0.1	2.2	0.2	0.26
3rd Operation					
Volume B	25.8	3.6	122.4	23.1	0.001
Histopathology B	2.2	0.2	2.4	0.3	0.72
4th Operation					
Volume C	32.7	6.0	109.2	12.8	0.001
Histopathology C	2.7	0.2	2.6	0.2	0.9

Discussion

Endometriosis is a common disease with a prevalence of 10% in reproductive women; the prevalence of this disease is higher in infertile women (25-40%). Although it is a benign disease, it may cause severe peritoneal adhesions, pelvic distortions, secondary dysmenorrhoea, dyspareunia and infertility (42, 43). Various therapies have been used in an attempt to treat endometriosis, including ovarian suppression therapy, surgical treatment, or a combination of these strategies. The knowledge of the high local oestrogen effects on the severity of disease leads to medical hormonal suppression via progesterone, oestrogen and progesterone combinations, GnRH analogues, danazole and gestrinone (44). Aromatase inhibitors like letrozole and anastrazole are used to inhibit oestrogen formation from androgenic precursors; TNF-α inhibitors are used to inhibit the mechanism of inflammation which leads to propagation of disease. Anti-angiogenic substances and inhibitors of matrix metalloproteinase are used to inhibit further invasion of endometriosis through natural surfaces (44). However, the adverse effects of these treatments limit their long-term use. In addition, recurrence rates after the cessation of therapy are high, and the treatments have had no benefit in endometriosis-associated infertility (45). Therefore new medical treatments, which are as effective as hormone treatments with an improved or no side effect profile, are needed.
Melatonin is a broad-spectrum antioxidant and therefore may interfere with the oxidative stress seen in endometriosis (31). The impact of melatonin on the development of endometriosis through oxidative stress balancing was elegantly demonstrated, by a study in which pinealectomised rats with induced endometriosis developed lesions of a volume that was statistically significantly higher than seen in a control group in which

Table 2. Summary of results within the melatonin group and control groups

		2nd Operation Mean ± SEM		3rd Operation Mean ± SEM		4th Operation Mean ± SEM		p
20 mg/kg/day Melatonin	Volume	108.2	17.5	25.8	3.6	32.7	6.0	0.001
	Histopathology	1.7	0.1	2.2	0.2	2.7	0.2	0.009
Control Group	Volume	124.5	14.8	122.4	23.1	109.2	12.8	0.20
	Histopathology	2.2	0.2	2.4	0.3	2.6	0.2	0.14

endometriosis was induced with no previous pinealectomy (46). In the pinealectomy group, the level of MDA was statistically significantly higher, and SOD and CAT activity was statistically significantly lower. After the administration of melatonin, lesions in treated animals became similar to those seen in the controls.

Similarly, daily melatonin administration led to a significant reduction in the volume and weight of endometriosis-like lesions in controlled animal studies. Molecular markers of oxidative stress such as malondialdehyde (MDA) were statistically significantly reduced, and antioxidant activity, measured by SOD and catalase (CAT), was statistically significantly increased (35, 47).

Melatonin may also regulate endometriosis by interfering with MMP activity. In the mouse model it was shown that melatonin down-regulated proMMP-9 and MMP-3 expression and activity, and enhanced the expression of a natural inhibitor of MMPs known as TIMP-1 (tissue inhibitors of metalloproteinases) in a dose-dependent manner. The preventive and therapeutic action in endometriosis-like lesions was confirmed, with an increased apoptotic index (34, 48).

Our study showed that treatment with melatonin effectively regressed endometriotic explants in a rat model. A homologous rat endometriosis model was used in our study. Endometriosis was induced surgically under anaesthesia, as proposed by Vernon and Wilson with modifications by Uygur et al. and by us (35, 37, 38, 40, 49).

Oophorectomy was performed to inhibit fluctuation of endogenous oestrogen levels with respect to physiology of rats. Then, high dose of continuous oestrogen (50 μg/kg; twice a week) treatment was started; the 20 mg/kg/day melatonin dose was only given 10 rats which were randomly chosen. After two weeks of melatonin treatment, an additional two weeks of continuous oestrogen was administered to assess the endometriotic recurrence in the melatonin group. During the study, endometrial lesion volumes at the fourth and sixth weeks were decreased in the melatonin group when compared to those in the control group without histopathological differences between groups. The results were consistent with the previous studies in respect to lesion volumes and histology (35, 47).

Furthermore, when the melatonin group was analysed within itself, lesion volumes were insignificantly changed after six weeks, which means that melatonin might lower the recurrence rate. Also, histopathological scores were significantly improved in the melatonin group at the end of the sixth week in comparison with standard endometriotic lesions in the second week with an insignificant increase in the fourth week.

In conclusion, melatonin seems to be a promising non-hormonal treatment agent with obvious effects to minimise endometriosis and with probable effects to reduce the recurrence or to increase the lesion differentiation. In our study, the volumes of surgically induced endometriotic foci were decreased and histopathological scores were significantly improved with melatonin treatment, in spite of high doses of continuous exogenous oestrogen administration throughout the study period.

However further studies using different doses of melatonin or different animal models are required to confirm the safety and efficacy of melatonin.

Ethics Committee Approval: *This study was approved by the Experimental Animals Ethics Committee of Yeditepe University Medical Faculty. All experiments were performed in compliance with international guidelines on the ethical use of animals.*

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Awareness and use of folic acid among reproductive age and pregnant women

Üreme çağında ve gebe kadınlarda folik asit hakkında bilgi düzeyi ve kullanım durumu

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Abstract

Objective: Folic acid supplementation during the pre-conception period and first trimester of pregnancy reduces the incidence of neural tube defects (NTDs). In this study, our aim is to investigate knowledge and use of folic acid among women attending our clinic.

Material and Methods: Between January 2012 and June 2012, 817 participants, consisting of 345 pregnant and 472 non-pregnant women, were enrolled in this survey. A questionnaire including socio-demographic information, knowledge and use of folic acid was applied.

Results: 48.2% of participants were aware of folic acid for the prevention of congenital anomalies. Knowledge and use of folic acid increase with socio-economic status and educational level. Participants who were already knowledgeable about folic acid cited health care professionals as common sources of information. Although 88.2% of the pregnancies were planned among the currently pregnant women, only 14.2% of them stated that they had used folic acid in the pre-conception period. The use of folic acid during the first trimester among pregnant women was 48.6%. Furthermore, 18.4% of participants had not used folic acid and 29.3% of them had not remembered whether they had or not. Even though 94.4% of health care professionals had heard about folic acid, 28.3% reported that they had used folic acid before pregnancy.

Conclusion: It is thought that there is a relatively high incidence of NTD in Turkey, which is due to inadequate information about NTDs and the use of folic acid. Primarily health care professionals such as midwives, nurses and family physicians should aim to inform all reproductive age women about folic acid for the prevention of NTDs, who should be encouraged to take the supplement when planning pregnancy. (J Turkish-German Gynecol Assoc 2013; 14: 87-91)

Key words: Folic acid, neural tube defect, pregnancy

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Özet

Amaç: Perikonsepsiyonal dönemde ve 1. trimester boyunca folik asit desteği nöral tüp defektini (NTD) azaltmaktadır. Çalışmamızda hastanemize muayene için gelen üreme çağındaki hastalar ile birlikte gebe kadınların folik asit kullanımını ve bunlara etki eden faktörleri araştırmayı amaçladık.

Gereç ve Yöntemler: Ocak 2012- Haziran 2012 tarihleri arasında hastanemiz Kadın Hastalıkları ve Doğum polikliniğine gelen 817 hastaya (345 gebe, 472 gebe değil) sosyodemografik bilgiler ve folik asit kullanımı ile ilgili soruları içeren bir anket uygulandı.

Bulgular: Hastaların % 48.2'si folik asidin doğumsal anomalileri önlediğini bildirmiştir. Folik asit hakkındaki bilgi ve kullanımı ekonomik düzey ve eğitim düzeyi ile artmaktadır. Katılımcılar folik asit hakkındaki bilgileri en fazla sağlık çalışanlarından öğrendiklerini belirtmiştir. Gebe katılımcılar arasında, gebeliklerin % 88.2'i planlı olmasına rağmen sadece % 14.2'de prekonsepsiyonel dönemden başlayarak folik asit kullanılmıştır. Gebeliğin ilk 3 ayında folik asit kullanım oranı ise % 48.6'dır. Gebeliğin hiçbir döneminde folik asit kullanmayanlar % 18.4, kullanıp kullanmadığını bilmeyenler ise % 29.3'dür. Çalışmaya katılan sağlık çalışanlarının % 94.4'ü folik asidi bilirken, bunların % 28.3'de gebelik öncesinde folik asit kullandıkları saptandı.

Sonuç: Ülkemizde NTD sıklığının göreceli olarak yüksek olduğu düşünüldüğünde doğurganlık dönemindeki kadınların NTD ve folik asit kullanımı hakkında yeterli bilgiye sahip olmadıkları, bu konuyu iyi bilen sağlık çalışanlarının ise gebelik öncesi dönemde yüksek oranda folik asit kullanmadıkları görülmüştür. Gebelik öncesi folik asit kullanımının azlığı, üreme çağındaki kadınların folik asitle ilgili daha ayrıntılı bilgilendirilmeleri gerektiğini düşündürmektedir. Bu amaçla öncelikle ebe, hemşire ve aile hekimleri gibi hastaların ilk etapta ulaşacağı sağlık personelinin hastaları bilgilendirme yönünde eğitilmesi ve bu kişilerin, doğurganlık çağındaki tüm kadınlara NTD'nin önlenmesinde folik asidin etkisinin duyurulması için çalışmalar yapmaları gerekmektedir. (J Turkish-German Gynecol Assoc 2013; 14: 87-91)

Anahtar kelimeler: Folik asit, nöral tüp defekti, gebelik

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Introduction

Folic acid, also called folate in its nature form, is a form of water soluble vitamin B9. It is found in green leafy vegetables (broccoli, spinach, lettuce, romain lettuce) and some fruits (orange, banana). It is the coenzyme that transfers single carbon groups for nucleic acid and amino acid metabolism. Folic acid has an important role in DNA/RNA synthesis, amino acid transformation, the formation of red blood cells, and the formation and maintenance of the body cells. Folic acid require-

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ments increase during periods of rapid growth and division of the body's cells throughout life (1, 2).
The term neural tube defects (NTDs) refers to any malformation of the embryonic brain and/or spinal cord. During the embryogenesis period, the brain and spinal cord originate from the neural tube. Methionine is essential for neural tube development. In folic acid deficiency, homocysteine cannot be converted to methionine; as a result, homocysteine levels increase, and methionine levels remain lower. Due to increased homocysteine levels in folic acid deficiency and cardiovascular diseases, NTDs like anencephaly and spina bifida may develop. The failure of neural tube closure prior to the end of the fourth week of embryogenesis causes NTDs. Genetic and environmental factors play an important role in the aetiology of NTDs (3, 4).
Studies show that the supplementation of 0.4 mg folic acid during the periconceptional period prevents 50-70% of NTDs (5, 6). Normal dietary intake meets half of the daily folic acid requirement (7). As a consequence, since the early 1990s, health authorities in many developed countries have recommended that all women planning to become pregnant should consume additional dietary and supplementary folic acid periconceptionally (8-12).
We aimed to determine awareness, knowledge and use of folic acid and the factors that affect folic acid intake among Turkish women.

Material and Methods

The cross-sectional survey was conducted at the Department of Obstetrics and Gynecology, Antalya Teaching and Research Hospital, from January 2012 to June 2012. Participants consisted of 345 pregnant women admitted to the antenatal clinic, and 472 non-pregnant women including hospital staff and patients admitted to the gynaecology clinic with a variety of complaints. A questionnaire that consisted of 19 questions was applied by doctors for randomly selected women aged 19-45 years. Demographic characteristics (age, marital and socioeconomic status, educational level), obstetric history (previous pregnancy number, child with neural tube defect), knowledge about folic acid and NTD, the use of folic acid during the periconceptional period, source of information regarding folic acid were questioned. Awareness was assessed by respondents having 'heard or read' about folic acid. Knowledge about folic acid was defined as knowing that folic acid prevents NTDs. Data were analysed using SPSS version 11.0 (SPSS Inc. Chicago, USA). The Ethical Committee of our hospital approved the study. All participants provided written informed consent.

Results

The total number of women included in the study was 817. The mean age of the women was 32.4 (SD±8.19) years. 92.9% of participants were married and 22% (180 women) were health care professionals. 60% of pregnant women were multiparous compared to 75% of non-pregnant women. Pregnancies were planned in 88.2% of the currently pregnant women; the mean gestational age at enrolment was 27.5 (SD±9.7) weeks. Demographic characteristics of the women participating in the study are given in Table 1.
Three hundred and forty five of the women (42.2%) reported that they had heard about folic acid and 394 (48.2%) of them stated that folic acid could prevent birth defects; however, only 150 (18.3%) women could specify its role in preventing NTDs. Knowledge and the use of folic acid was associated with educational level (Figure 1). 18.4% of participants stated that they had not used folic acid at any time during pregnancy and 29.3% of them did not remember whether they had used folic acid or not (Figure 2). Knowledge of folic acid was lowest among women aged 35-49 years (30.8%) and highest among women aged 25-34 years (45%). The proportion of women using periconceptional folic acid was 21.1%. Doctors were the most frequent source of information (76.6%), but other sources of information were relatives/friends (19.9%) and the media (3.5% -television/magazine/internet).
Among the pregnant women, 225 (65.7%) of them reported that they had heard about folic acid; initiation of folic acid intake at least one month before conception and during the first trimester was seen in 14.2% (49 women) and 48.6% (168 women), respectively. 94.4% of health care professionals were aware of folic acid; nevertheless, only 28.3% reported that they had used folic acid before pregnancy.
12 of the participants had a history of a foetus with NTD. Among these, only 25% of them had taken folic acid before pregnancy, 75% of them had never used the supplement or had been informed about the need for supplementation of folic acid during pregnancy.

Table 1. Demographic characteristics of the women

	n	%
Age (years)		
<25	159	19.4
25-34	356	43.5
>35	302	37.1
Education		
Illiterate	14	1.9
Primary	266	32.5
Secondary	126	15.4
High school	166	20.3
University and above	245	29.9
Currently pregnant		
Yes	345	42.2
No	472	57.8
Marital status		
Married	759	92.9
Single	58	7.1
History of NTD		
Yes	12	1.5
No	805	98.5

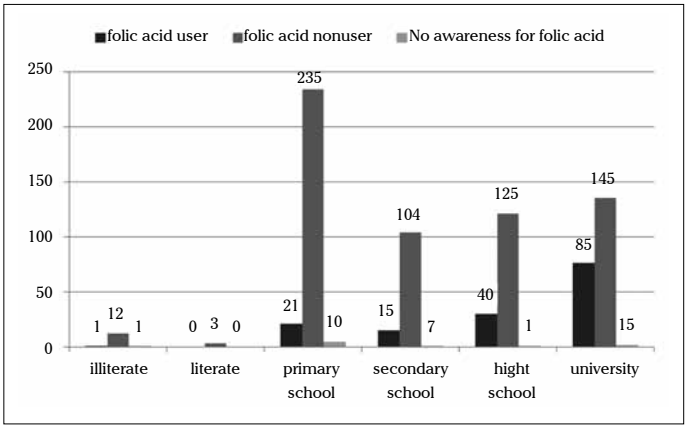

Figure 1. Distribution of educational level among folic acid users

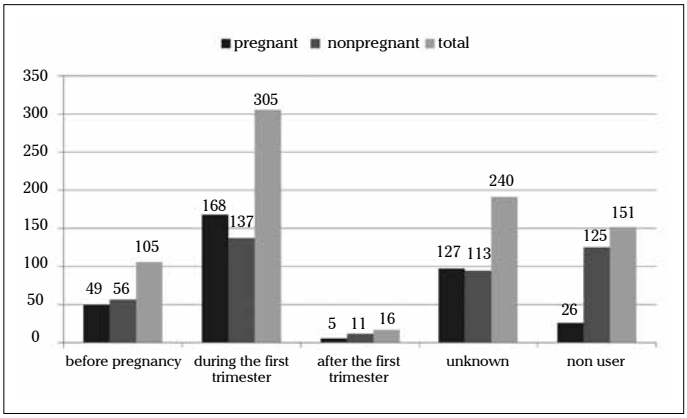

Figure 2. Distribution of folic acid intake among participants

Discussion

The prevalence of NTD varies in the ranges 0.4-1.6, 2-4, 2.9-5.2, 4-9 per 1000 live births in the European countries, in the USA, in the Middle Eastern countries, and in Turkey, respectively (13, 14). To increase the awareness of genetic disorders among health care personnel, the Turkish Ministry of Health issued a guideline in 2002. This guideline recommends daily folic acid supplement for all women of childbearing age before conception and throughout the first trimester (15). When the economic situation and high incidence of NTD in Turkey is considered, the prevention of these malformations is particularly important. In this study, levels of awareness, knowledge, and the use of folic acid periconceptionally were found to be 42.2%, 18.3% and 21.1%, respectively. Socio-economic and educational level caused a geographical distribution regarding awareness of folic acid in Turkey. A cross-sectional study conducted in Konya reported that only 22% of participants were aware of folic acid, and that 13% of them indicated knowledge about the relationship between folic acid supplementation and NTD prevention (16). Another study conducted in Edirne reported that 18.6% of the subjects had heard of folic acid, but only 6.4% of them knew about its preventive role against NTDs and 1% of them had taken folic acid prior to conception (17). A pilot study on a community-based training programme highlighted the importance of informing the population about folic acid. A regional campaign was organised to create awareness among women in Izmir. Before the campaign was carried out, 18% of women had heard of folic acid; their level of knowledge increased after the campaign (18). Another cross-sectional study that was carried out in Kayseri reported the awareness of folic acid as 46.3%, the use of folic acid periconceptionally as 12.2%, and knowledge about folic acid as 6.3% (19). The trend in other countries is different, with reports of folic acid awareness rate as high as 53.7% in Arabia, 24.4% in Thailand, 35.8% in China, 95% in Canada (20-23).

In total, 65.7% of pregnant women in our survey had heard of folic acid, and 14.2% of them had taken folic acid during the whole protective period. However, this correct folic acid taking rate during the recommended period in pregnant women was lower than the rate from developed countries (24-26). Another considerable finding in our study was that the use of folic acid among health care professionals (28.3%) preconceptionally was lower than expected. This suggests that an awareness of folic acid does not necessarily increase periconceptional use of folic acid supplements.

The risk of NTD recurrence in subsequent pregnancy is 2-3% (5). No recurrence of NTD was reported in a study conducted on 190 women with a previous pregnancy with NTD who received 5 mg folic acid per day during the periconceptional period. This study also pointed out that NTD recurrence was seen in 4.04% of the patients who had not used folic acid (27). Another study reported a rate of 72% reduction in recurrent risk of NTDs in women who received 4 mg of folic acid per day during the periconceptional period (8).

Due to the fact that NTDs occur during the 22^{th}-28^{th} days of the embryonic period, before most women even know that they are pregnant, the initiation of folic acid supplements after the first month of pregnancy is too late to prevent NTDs. Thus, in 1992, the US Public Health Service recommended that all women capable of becoming pregnant consume at least 400 micrograms (0.4 mg) of folic acid per day preconceptionally and continue throughout the first trimester (28). For women who had a previous pregnancy with an NTD, folic acid supplementation of 4 mg per day during the same period is recommended (29). Despite these recommendations and national campaigns, periconceptional intake of additional folic acid remains very low in many countries (30, 31).

In recognition of the need to decrease the risk of NTDs, fortification programs were mandated in both the United States and Canada in 1998. The United States made folic acid fortification mandatory for bread and grain products at 0.14 mg folic acid/100 g flour and 0.24 mg/100 g pasta (32). In Canada, fortification of flour and pasta was set at 0.15 mg folic acid/100 g and 0.20 mg/100 g, respectively. This fortification is estimated to meet 25% of the recommendation of folic acid (33). It is known that to improve public health, the enrichment of foods with folic acid is a very important approach in order to reduce the incidence of NTD. However, fortified foods are not sufficient for many women; also, there is no food fortification with folic acid in many countries, like Turkey. Supplementation of vitamins is needed to achieve the recommended daily dose (34).

Despite numerous health benefits of folic acid supplementation, emerging evidence in recent years suggests that increased population exposure to folic acid may also have a negative impact with respect to certain developmental and degenerative disorders. For example, much attention is currently focused on the role of folic acid fortification augmenting colon cancer risk (35). A similar, perhaps unexpected negative effect has also been shown during pregnancy. Synthetic folic acid saturates human dihydrofolate reductase, leading to unmetabolised folate in circulation, possibly masking irreversible pernicious anaemia seen in B_{12} deficiency. Low maternal vitamin B_{12} and high folic acid status during pregnancy may contribute to insulin resistance and obesity in offspring (36).

The incidence of NTD is considerably higher in our country. These abnormalities are substantially preventable by the encouragement of folic acid usage. When comparing the studies from Turkey and other countries, a lack of information and the use of folic acid drew attention. Further efforts are needed to inform the population and promote the optimal use of folic acid supplements. Therefore, health care professionals should aim to instruct the effects of folic acid to all women of reproductive age for the prevention of NTDs. Additionally, it may be appropriate to inform patients who attend health care centres and distribute comprehensive brochures about folic acid. Public health campaigns can be arranged to announce the effect of folic acid on preventing NTDs. These campaigns should address health care professionals, and all women and men of reproductive age. Health professionals should consult all reproductive aged women about the importance of folic acid for the prevention of NTDs. Due to the fact that essential amounts of folic acid cannot be achieved by diet alone, daily folic acid supplementation should be given to women during the periconceptional period.

Ethics Committee Approval: *Ethics committee approval was received for this study.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

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Impact of earthquakes on sex ratio at birth: Eastern Marmara earthquakes

Depremlerin doğumda cinsiyet oranına etkisi: Doğu Marmara depremleri

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Abstract

Objective: Previous reports suggest that maternal exposure to acute stress related to earthquakes affects the sex ratio at birth. Our aim was to examine the change in sex ratio at birth after Eastern Marmara earthquake disasters.

Material and Methods: This study was performed using the official birth statistics from January 1997 to December 2002 – before and after 17 August 1999, the date of the Golcuk Earthquake – supplied from the Turkey Statistics Institute. The secondary sex ratio was expressed as the male proportion at birth, and the ratio of both affected and unaffected areas were calculated and compared on a monthly basis using data from gender with using the Chi-square test.

Results: We observed significant decreases in the secondary sex ratio in the 4th and 8th months following an earthquake in the affected region compared to the unaffected region (p= 0.001 and p= 0.024). In the earthquake region, the decrease observed in the secondary sex ratio during the 8th month after an earthquake was specific to the period after the earthquake.

Conclusion: Our study indicated a significant reduction in the secondary sex ratio after an earthquake. With these findings, events that cause sudden intense stress such as earthquakes can have an effect on the sex ratio at birth.

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Key words: Earthquakes, sex ratio at birth

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Özet

Amaç: Önceki çalışmalarda, depremler ile ilişkili maternal akut stres maruziyetinin doğumda cinsiyet oranına etkisi bildirilmişti. Bu çalışmada amacımız Doğu Marmara deprem felaketlerinden sonra doğumda cinsiyet oranındaki değişimi incelemekti.

Gereç ve Yöntemler: Bu çalışma Türkiye İstatistik Kurumu'ndan (TUİK) sağlanan Ocak 1997 ve Aralık 2002 yılları arasında, -17 Ağustos 1999 Gölcük depreminden önce ve sonra- resmi doğum istatistikleri kullanılarak gerçekleştirildi. Sekonder cinsiyet oranı, doğumda erkek oranı olarak ifade edildi ve sekonder cinsiyet oranı, etkilenmiş ile etkilenmemiş bölgelerin ikisinde de cinsiyet verileri ve ki-kare testi kullanılarak aylık bazda hesaplandı ve karşılaştırıldı.

Bulgular: Biz Gölcük depreminden sonraki 4. ve 8. aylarda etkilenmiş bölgede, etkilenmemiş bölge ile karşılaştırıldığında, sekonder cinsiyet oranında anlamlı azalma olduğunu gözledik (sırası ile p= 0.001 ve p= 0.024). Deprem bölgesinde, depremden sonraki 8. ayda sekonder cinsiyet oranında gözlenen azalma deprem sonrasına özgü idi.

Sonuç: Çalışmamız deprem sonrası sekonder cinsiyet oranında anlamlı azalma olduğunu gösterdi. Bu bulgular ile deprem gibi ani yoğun strese neden olan olayların doğumda cinsiyet oranı üzerine etkili olduğu göz önünde bulundurulmalıdır.

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Anahtar kelimeler: Depremler, doğumda cinsiyet oranı

Geliş Tarihi: 18 Nisan 2013 **Kabul Tarihi:** 09 Mayıs 2013

Introduction

The sex ratio of females to males at birth is called the secondary sex ratio; the incidence in humans is known to be approximately 1:02 to 1:06 (1). The secondary sex ratio (SSR) is associated with many factors that vary between communities and over the years. In the literature, there have been studies indicating an interaction between the ratios of race, geographical region, the environmental temperature, seasonal changes, and social and country-specific factors such as socio-economic development (2-6). In addition, earthquakes, floods, famine periods, environmental disasters, wars, natural or man-made disasters, such as intense psychological and physical stress caused by the individual, and the society's general health status of pregnant women and pregnancy outcomes can influence the sex ratio at birth, which are likely to change (7-10). Fukuda et al. (7), Saadat et al. (11), Torch et al. (12), and Tourikis et al. (13) investigated the Kobe (Japan) earthquake, the Ban (Southern Iran) earthquake, the Tarapaca (Chile) earthquake, and the Zakynthos (Greece) earthquake, respectively, and reported a decrease in male births compared to female births in the period after the earthquake; in other words there was a decrease in the female secondary sex ratio. These findings suggest a better adaptation process in humans, as well as the size of the damage caused by an earthquake on society. The effects of both natural and man-made disasters can occur many years later, and babies born during stressful periods may encounter increased health problems and increased mortality rates in the future (14). The first sign of deterioration in public health at birth after natural disasters over the years may be a change in sex ratios.

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Movement in the North Anatolian Fault Zone caused two devastating earthquakes in the Eastern Marmara Region. The first earthquake epicentered on Gölcük-Izmit city (17 August, 1999) and was classified as one of the world's six deadliest earthquakes in the 20th century; 87 days later, a second earthquake hit Düzce city (12 November, 1999). According to official records, 18,287 people died and 46,857 were injured. A total of 108,861 buildings, schools and workplaces were destroyed by earthquakes, 1.1 million people became homeless and, especially due to inadequate rescue and support services, communication and shelter systems, the whole nation was shaken and more than 16 million people were impacted (15).
In this study, we evaluated the sex ratio at birth before and after the Eastern Marmara earthquakes compared to the control region.

Material and Methods

This study was performed using the official birth statistics in January 1997 and a total of 72 months up to December 2002, which were supplied from Turkey Statistics Institute (Turkstat). The secondary sex ratio was expressed as the male proportion at birth, i.e. males/females.
The movements of the North Anatolian Fault Zone caused the Gölcük-Izmit earthquake (17 August, 1999, magnitude on Richter scale 7.8, on Mercalli scale category X: disastrous) and the Düzce earthquake (12 November, 1999, magnitude on Richter scale 7.5, on Mercalli scale category IX: ruinous) (16). Comparisons before and after the earthquake due to the size of the impact of Gölcük earthquake were considered as a reference point. The secondary sex ratio at birth in the affected area (study group) in the eastern Marmara region earthquakes was calculated on a monthly basis using gender data. The unaffected area (control group), was accepted as a location approximately 400-450 km from the shock during the earthquake, in the Mediterranean region, without any destruction or death, and ratios were calculated on a monthly basis using data. In order to demonstrate factors such as changes in season and agricultural work that may affect the results of our study, we identified the region on the same meridian that was unaffected by the earthquake. The Western Anatolia, the Black Sea Region, and the Mediterranean Region were deemed appropriate as unaffected regions since all of the Western Anatolia was affected by the earthquake, the Black Sea region was on the same earthquake fault line, and the Mediterranean region had a cosmopolitan structure, but the eastern Marmara region has heavy industrialisation and the revenue of the Mediterranean region was derived from tourism and agriculture. The effects of out-migration after the earthquake in the eastern Marmara region and the summer seasonal movements causing an increase in population in the Mediterranean region according to statistics could not be assessed in the study. All data were analysed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) and Statistica Program 5.0 (StatSoft Inc., Tulsa, OK, USA). Statistical analysis of the sex ratio was performed using the Chi-square test. The significant differences were defined as a p value less than 0.05.

Results

During the five-year period examined in this study, 800,742 male and 762,356 female babies formed a total cohort of 1,563,098; the overall SSR was 51.22. Of the deliveries, 627,743 took place in the Eastern Marmara region and 935,355 took place in the Mediterranean region; the SSR in eastern Marmara and the Mediterranean regions were 50.74 and 51.55 respectively (p=0.959). Compared to the previous 12 months and the 12 months immediately after the earthquake, while the birth rate decreased (2.66%) in the earthquake area, the rate increased (4.42%) in the unaffected region.
Sex changes in rates between regions were evaluated monthly and revealed a significant decrease in the secondary sex ratio in the 4th month (December, 1999) and 8th month (April, 2000) following the Gölcük earthquake in the eastern Marmara region compared to the Mediterranean region (p=0.001 and p=0.024). The statistically significant change observed between the two regions in December was also observed in December 1998, December 2000 and December 2001. In fact, in both regions, there was a significant difference between December and January births. This result seems to be related to changes in seasonal population registers. The reasons for the difference observed in the Eastern Marmara region in December might be the legal 30 day notice period in Turkey for registration and the common tradition of birth registration of babies born in December to make their ages one year younger according to records. A significant increase was observed 3 months prior to the earthquake (May 1999) in the SSR only specific to that month (p=0.046). The statistically significant reduction in the secondary sex ratio observed in April 2000 was not observed in any other month during the study period. A pregnancy occurring after ovulation in August 1999 would be expected to be born in May 2000; in our study, we did not detect any significant changes in SSR in May 2000 (p=0.595). Also, no significant change has been observed in SSR since then (Figure 1). The decrease observed in male infants in the affected area in April 2000 when compared to male infants in April 2001 and April 2002 was significant (p=0.032 and p=0.049, respectively).

Discussion

Hansen et al. (17) have examined the effects of individual intense stress on SSR and revealed a decrease in SSR after such

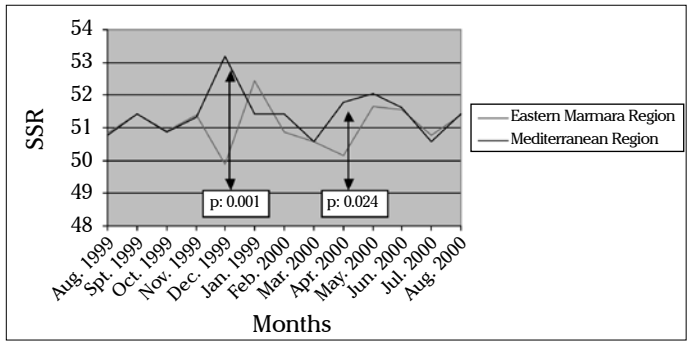

Figure 1. Sex ratio changes after earthquakes in regions on monthly basis

events leading to severe stress, such as a spouse's serious illness or loss of a child or a husband starting 12 months before conception onwards, including the periconceptional period in the first trimester. However, Khashan et al. (18) expanded their research by adding all births in the next 10 years in the cohort, and revealed a slight decrease that was not statistically significant in the birth sex ratio; Obel et al. (19) and Subbaraman et al. (20) in African-American patient groups have supported the evidence provided by Hansen et al. (17). Hansen et al. (17) and Obel et al. (19) calculated OR in patients with stress and identified ORs as 0.65 and 0.85, respectively. In these studies, the SSR of the control groups were 1 and 1.07, respectively.

Large earthquakes that may impair the mental health of an individual are a common cause of stress. After the Golcuk and Duzce earthquakes, post-traumatic stress disorder, major depression, panic disorder, obsessive-compulsive disorders, generalised anxiety disorder, phobias, and suicidal tendency were found to be increased and remain high for years (21). This can have a disastrous impact on mental health in pregnant women, and, after a large earthquake, minor psychiatric problems are seen in 1/3 of pregnant women (22). The possibility of shortening the duration of the pregnancy, abortion rate, preterm birth rate and twin pregnancy, the foetus brain development retardation, ear malformations, facial cleft abnormalities and changes in sex ratios at birth have been reported as a cause of severe psychological stress just before pregnancy or during pregnancy (17, 19, 20, 23-26).

Studies investigating the Kobe earthquake, the Ban earthquake, the Tarapaca earthquake, and the Zakynthos earthquake revealed a decrease in the secondary sex ratio in the period after the earthquake (7, 11-13). The ways in which psychological stress of the earthquake reduces the sex ratio at birth are controversial. The reason for the reduction of SSR might be a reduction of male fertilisation (reduction in the reflection of the primary sex ratio), or a higher loss of males than females after fertilisation (direct reduction in SSR). Support for the hypothesis of reduced male fertilisation can be found in the study by Fukuda et al. of the Kobe earthquake (7,27). In these studies, it has been shown that the stress, especially in people who have lost relatives and whose houses were destroyed in the earthquake, reduces coitus frequency, sperm motility for 2-9 months, and also a reduction in SSR nine months after the earthquake. Although unproven, Y-bearing sperm with loads that are lower than X oocyte achieve an advantage (28). While a decrease in coitus frequency results in the possibility of fertilisation coincident with late menstruation, due to thickening of cervical mucus associated with low sperm motility, the advantage resulting from the loss of Y-bearing sperm, and consequently the rate of progression is likely to decrease in the primary sex ratio. In this situation, because of the reduced male fertilisation, the SSR will change against males, and the male and female birth rates will be found to be closer to each other. High coitus frequency in Micronesia and low SSR related with thickened cervical mucus in clomiphene citrate cycles favour this hypothesis, while publications with no relation of SSR to the timing of intercourse, insemination time, and follicular phase length provide evidence against (29-32).

Trivers-Willard hypothesis suggests a favour of female reproduction in women with poor health status and male reproduction in women with good health status (33). At a time of scarce resources, because a boy has a high probability of growing faster and being more resource-consuming, as well as a high probability of loss before reaching reproductive age, instead of giving birth to a boy, a girl summarises the efficiency of this hypothesis. Despite strong evidence confirming this hypothesis in animals, it is not easy to achieve similar results in humans (34). In groups of patients with anorexia and bulimia, studies have indicated a decrease of male live births, sex ratios decreased secondary to diabetes in women, and the poor health status of mothers giving birth to female babies; this supports the idea (35, 36). In the study investigating the shortage of grain in China in 1959-1961 it was reported that adaptation to long-term effects results in a reduction of SSR (9). However, studies assessing the results of Dutch Hunger Winter of 1944-1945 or of the Cuban economic crisis have not produced evidence to confirm this hypothesis (37, 38). James reported that normal values of gonadal hormones like testosterone and oestrogen reflect the health status of women and men, and are associated with an increase in SSR; in contrast, high levels of gonadotrophic hormones (FSH, LH) reflect an unhealthy status and are associated with a decrease in the ratio in his research and reviews about sex ratios (39-41). Low SSR in people with dioxin, dibromochloropropane (DBCP) exposure, which have strong anti-oestrogenic and anti-androgenic effects on environmental pollution, are considered among the factors associated with endocrine disruptive chemicals, which support this view (30, 42). In industrialised North American and Northern European countries, increasing pollution leading to endocrinologic destruction has been suggested to be responsible for the decrease in SSR in recent decades (43).

Contrary to the hypothesis that secondary sex ratio variation reflects changes in the primary sex ratio, another hypothesis suggests that the secondary sex ratio decreases due to an increase in embryo/foetal loss after major disasters (44). In fact, we know that more male babies are lost in the early weeks of gestation. SSR in patients with miscarriage at 16-19 weeks of gestation, at 20 weeks of gestation, and delivery after 36 weeks of gestation were 248:100; 130:100; and 105:100, respectively (45). The answer of the question of whether the embryo loss rate increases after large earthquakes, however, is not yet clear. Catalano and Bruckner reported severe psychological stress after the 9/11 terrorist attacks, economic crises, and in collective dismissal issues, all of which reduce SSR (44, 46-48). In their study investigating the effects of stress after the 9/11 terrorist attack, they stated the reduction in SSR, not after 8, 9 and 10 months, but within the first 3 months, suggesting that this result is not related to fertilisation, but rather a greater increase in loss associated with male foetuses (46). According to this hypothesis, when the mother's health is in danger, weak male foetuses are sacrificed more by glucocorticoid levels compared with female foetuses. Hobel et al. have shown that after 20 weeks of gestation, after maturation of the foetal nervous system, foetuses are more susceptible to elevated maternal glucocorticoids, which was associated

with preterm birth (49). However, pregnancy is a process where sensitivity to the effects of stress from the outside is decreased. For example, during pregnancy, compared with a non-pregnant period, more corticotrophin releasing hormone (CRH) is needed for adrenocorticotropin hormone (ACTH) response (50). Also, pregnancy is characterised by decreased vascular response to epinephrine and norepinephrine infusion, reduced heart rate and blood pressure response to physical stressors (51). This insensitivity gradually increases in the following weeks of gestation (52). After the Northridge earthquake in California, US, in 1994, the emotional effects of earthquakes were found to be higher during early pregnancy and stress encountered in early pregnancy is shown to shorten the duration of pregnancy (53).

Another point that has to be explained for the foetal loss hypothesis is whether all of the foetuses are affected by stressor factors. Catalano et al. have shown in their study that weak foetuses are lost due to stress, or if they survive are not damaged, and such cohorts of individuals born later in their lives are not associated with increased morbidity and mortality (culled cohort hypothesis) (54). However, a cohort of babies born from damaged foetuses are associated with an increased morbidity and mortality risk in the future (damaged cohort hypothesis) (55, 56). The knowledge that such infants born during periods of stress have an increased risk of metabolic, cardiac and psychiatric illness in their adulthood and a reduced life expectancy found a place in the literature (14, 57, 58). As shown by Van den Berg et al., mortality rates are higher and the average life expectancy is 10 months shorter in patients >35 years of age in times of economic recession compared with patients born in times of good economic status (14). Similarly, the study by Dama has shown an inverse proportion of SSR with the future mortality rate (58). If stress has occurred as a consequence of a disaster like an earthquake affecting the whole society, these diseases can become a public health problem 10 years later, so this may be an early marker of decreased SSR. Another creation of a community health plan might be required in cohorts with decreased secondary sex ratios after the earthquake.

In our study, we saw a significant decrease in secondary sex ratio about 8 months later when comparing the affected region with the unaffected area. This reduction was specific to only one month. First, Fukuda et al. (7) reported a significant decrease in the sex ratio 9 months after the Kobe earthquake. In the following years, Saadat et al. (11) stated that the psychological tension and stress as a consequence of the Ban earthquake was associated with a reduction in the sex ratio 6-12 months after and Tourikis at al. (13) found a significant decline in the sex ratio within a two month period starting from the 11th month after the Zakynthos earthquake. Torch et al. (12) stated in their study investigating the Tarapaca earthquake that the effects of the earthquake were significant on foetuses exposed during the 2nd or 3rd months of pregnancy, which resulted in a decrease in the sex ratio during birth. Our results support the studies indicating that the abortion rate of male foetuses exposed to the earthquake during the first trimester was higher than that of female embryos and indicates that the interaction was highest in the first month after the earthquake. A reduction in SSR after smoke pollution in London and the Brisbane flood occurred 320 days after the disaster; however, we did not determine such a long effect (8). Another factor with an impact on our results was an increase in preterm birth rates. If male babies are lost and female babies are born prematurely, then the reduction in SSR should be prior to 9 months of pregnancy. We have not had the chance to access hospital-based records, so we do not have data about an increasing preterm birth rate. After the earthquake in Golcuk, aftershocks continued for months and almost 3 months later a second major earthquake occurred; if we keep this in mind, it is difficult to explain why eight months after the earthquake the SSR was significantly decreased in just one month. The normalisation of the SSR after the acute loss of weak embryos as a response to stressful events can be one of the explanations. Further studies are needed to determine the adaptation rate to sudden stressful events during pregnancy. However, other studies have examined changes in the sex ratio at birth after earthquakes, and it should be kept in mind that the difference in SSR was significant only for a short period of time or a few months. Tan et al. (25) evaluated the effects of earthquakes in pregnancy after the Wenchuan Earthquake (2008, China) and did not detect a significant difference in SSR. However, the selective abortion of female infants in China is a common practice; as a result, it is not easy to determine changes in the SSR.

The effects of fire and related air and sea pollution in a large petrochemical refinery located very close to the city centre after 1999 Golcuk Earthquake can be discussed. Similarly, the smoke and air pollution following the Seveso accident, such as Minamata methylmercury pollution incidents, and the subsequent exposure of chemicals has been shown to lead to a decrease in SSR (8, 59-61). Moreover, if this argument is correct, the decrease in SSR can be used as an indicator of environmental pollution leading to a change in the nature of effects, and can be used for general health to take action on this issue. However, the most interesting thing is that the study by Yang et al. (62) of people living around the petrochemical refinery in Taiwan, and the study by Saadat et al. (63) about people living in areas of oil and natural gas have shown an increase in SSR as a contrast. It should be kept in mind that the Gulf of Izmit is located in the epicentre of the earthquake, and was in the region of the country's most intense air and marine pollution for 25 years before the earthquake. It is difficult to separately reflect the effects of the earthquake and environmental pollution on the statistics.

In conclusion, our study indicated that, as a consequence of the probable abortion of male embryos after large earthquakes, a significant reduction in the secondary sex ratio has been detected for a short period of time. With these findings, events that cause sudden intense stress, such as earthquakes, can have effects on the sex ratio at birth.

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Recurrence of endometriosis; risk factors, mechanisms and biomarkers; review of the literature

Endometriozis rekürrensi; risk faktörleri, mekanizması ve biyomarkerlar; literatürün gözden geçirilmesi

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Abstract

Endometriosis has a wide clinical spectrum and induces a chronic inflammatory process. The incidence of endometriosis in women with dysmenorrhoea is up to 40-60%, whereas in women with subfertility is up to 20-30%. Recurrence of endometriosis varies greatly among different studies. The overall recurrence rates range between 6 to 67% according to the criteria that are taken into consideration. Which of the various reasons is more predictive for recurrence is still unclear and controversial. The main aim of post-operative medical treatment is suppressing ovarian activity leading to atrophy of endometriotic lesions. The success of treatment depends on the resorption of all residual visible lesions and the eradication of microscopic implants. The recurrent lesions might originate from residual lesions or from de novo cells. Determining risk factors for recurrence may allow the identification of subgroups at risk for disease control. Potential biomarkers for recurrence could also maintain targeted therapy.

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Key words: Endometriosis, recurrence, endometrioma, cystectomy

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Özet

Endometriozis geniş bir klinik spektruma sahiptir ve kronik inflamatuvar bir süreç oluşturur. Dismenoresi olan kadınlarda endometriozis insidansı %40-60 iken, subfertil kadınlarda %20-30 arasındadır. endometriozis rekürrensi değişik çalışmalarda farklı sonuçlar sergiler. Dikkate alınan kritere göre rekürrens oranı %6 ile 67 arasında değişir. Rekürrens için birçok farklı nedenden hangisinin daha prediktif olduğu kesin değildir ve tartışmalıdır. Post operatif medikal tedavinin amacı ise overyan aktiviteyi suprese etmektir ve böylece endometriotik lezyonlarda atrofi sağlanır. Tedavinin başarısı tüm görülebilir rezidüel lezyonların ve mikroskobik implantların eradikasyonuna bağlıdır. Rekürren lezyonlar rezidüel yapılardan veya de novo hücrelerden kaynaklanabilir. Rekürrens için risk faktörlerini bilmek hastalığın kontrolü için subgrupların tanınmasını sağlar. Rekürrens açısından anlamlı potansiyel biyomarkerlar ise hedeflenmiş tedavinin temelini oluşturur.

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Anahtar kelimeler: Endometriozis, rekürrens, endometrioma, kistektomi

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Introduction

Endometriosis is the presence of endometrial tissue with glands and stroma outside the uterus. Although endometriosis is a benign disease, its clinical spectrum varies widely, independent from its severity; endometriosis induces a chronic inflammatory process (1). It is hard to know the exact prevalence of endometriosis because surgery is needed for accurate diagnosis and asymptomatic patients may decrease the prevalence (2). However, according to the available literature, the incidence of endometriosis in women with dysmenorrhoea is approximately 40-60%, whereas in women with subfertility it is up to 20-30% (3). Surgery is widely used for endometriosis treatment, but a significant risk of recurrence and unsolved symptoms make the disease the third most common cause of hospitalisation in the United States (4).

Recurrence rates

Endometriosis is a distinct entity that has an important role on morbidity and financial cost. Recurrence is frequently seen in patients with endometriosis and varies greatly among different studies. The overall recurrence rates range between 6 to 67% according to the criteria that are taken into consideration (5, 6). The discrepancy in the recurrence rate of endometriosis depends on a number of issues. Initially, the definition that was used for recurrence presents variations among studies; it might be expressed as pelvic pain described by the patient (dysmenorrhoea, dyspareunia) or clinical criteria (pelvic mass, nodulations on examination or imaging) or persistence in the situation of infertility. In a study by Vignali et al. (7) the reported recurrence rates for pain in 3- and 5-year periods were 20.5 and 43.5%, respectively. The clinical recurrence rates for those periods were 9 and 28%, respectively

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(7). Fedele et al. (8) reported the rate of recurrence for dysmenorrhoea as almost 30% after the end of a one-year period following laparoscopic surgery. Exacoustos et al. (9) defined recurrence as cysts with a diameter of more than 10mm and found that pain was a realistic determinant factor for surgery, and that 76% of patients who had a recurrence suffered from pain-related symptoms. The recurrence rates for pain and pain-related symptoms are higher than the clinical recurrence rates detected by sonographic findings (10). Secondly, the duration of follow-up period after surgery influences the rate of recurrence, as expected. It has also been reported that the average two-year recurrence rate is 19.1% (from 23 studies), and the five-year recurrence rate ranges between 20.5 and 43.5% (10). Busacca et al. evaluated 144 recurrences of endometriosis from 1106 women diagnosed with endometriosis and found four-year recurrence rates as 24.6, 17.8, 30.6 and 23.7% for ovarian, peritoneal, deep ovarian and peritoneal endometriosis, respectively. The rates of recurrence increased as time passed after surgery and the 8-year recurrence rates were 42, 24.1, 43.4 and 30.9%, respectively (11). Parazzini et al. (12) reported that the two-year recurrence rates for stage I and II endometriosis as 5.7%, whereas it was 14.3% for stage III and IV endometriosis.

Risk factors for recurrence

Although various reasons have been proposed as risk factors, it is still unclear and there is a controversy surrounding for which is more predictive for recurrence. Li et al. (13) followed-up 285 patients for a minimum of 36 months for recurrence after conservative surgery for endometriosis and reported that a history of endometriosis surgery, bilateral pelvic involvement of endometriotic lesions, left-sided endometrioma, tenderness, nodularity at cul-de-sac, post-operative high revised American Fertility Society (rAFS) scores, and younger age were all risk factors for recurrence. Liu et al. (14) followed 710 patients after endometriosis surgery for an average of 22.4 months and defined the recurrence of endometrioma as the presence of ovarian cysts 3cm in diameter for more than two consecutive menstrual cycles, and the recurrence of dysmenorrhoea as pain recurring after surgery with the severity score equal to or higher than that before surgery. They found that previous surgery, previous medication usage, younger age at surgery and total rAFS score were all risk factors for endometrioma recurrence; however, the total rAFS score was the only identified risk factor for dysmenorrhoea recurrence. For both recurrences, the hazard rate was constant in the first 28-30 months after surgery, but the hazard rate increased dramatically after that period.

After the evaluation of 121 patients following conservative laparoscopic surgery, Ghezzi et al. (15) reported that the left sided endometriomas recur more frequently, whereas Jones and Sutton (16) found that bilateral cysts were more likely to recur than single cysts after the evaluation of 73 patients following consecutive laparoscopic ablation. Saleh and Tulandi (17) evaluated 231 patients after the laparoscopic excision of endometrioma cysts and found that the reoperation rate was higher in women with larger cysts; Koga et al. (18) also reported similar findings after following-up 224 patients post-operatively for a minimum of 2 years. Busacca et al. (5) followed-up 366 patients after laparoscopic endometrioma excision and reported that the advanced stage disease and previous surgery for endometriosis were two risk factors for recurrence and that the rate of recurrence increased with an extended follow-up period; they also found that the cyst recurrence was mostly associated with the pain recurrence. Porpora et al. (19) followed-up 166 patients after laparoscopic endometrioma cystectomy and found that prior surgery, pelvic adhesions and high rAFS score were all negative prognostic factors for recurrence. Fedele et al. (20) evaluated 47 symptomatic bladder endometriosis patients and found that the radicality of surgery was important for recurrence. Vignali et al. (7) reported that the completeness of the first surgery was a prognostic factor for recurrence after a 12 month follow-up period in 115 symptomatic patients who were operated upon for deep infiltrating endometriosis. Parazzini et al. (12) reported that the advanced stage disease at initial surgery had a higher recurrence rate and Busacca et al. (11) showed deep endometriosis, younger age, stage 3 or 4 disease and time passed after surgery were all risk factors for recurrence.

Pregnancy after surgery has been stated as a protective factor for recurrence by many studies. Pregnancy may suppress the activation and growth of endometriotic lesions and also inflammation caused by endometriotic lesions with regard to the increased progesterone levels (10). Table 1 shows the risk factors for recurrence.

Preoperative medical therapy

Preoperative medical therapy before surgery in endometriosis may increase the risk of recurrence. Koga et al. (18) followed-up 224 patients for a minimum of 2 years post-operatively after laparoscopic ovarian endometrioma excision. They did not routinely administer pre- or post-operative medical treatment; this was only used because of the special needs of patients. 65 patients continued their medical treatment until the surgery and the average pre-operative medical treatment duration was 9.7 months. They concluded that the previous medical treatment for endometriosis caused significantly higher recurrence rates (18). That may be as a result of the endometriotic lesions being masked during the surgery because of the previous usage of medication. Moreover, medical treatment may alter some genomic processes of endometriotic lesions, and suppress normal eukaryotic cells. This may lead to an increase in dyskaryotic cells in the endometriotic implants by negative selection (21). Muzii et al. (22) also reported that pre-operative Gonadotropin Releasing Hormone (GnRH) agonist treatment did not provide any surgical improvement and did not affect recurrence rates.

Postoperative medication and endometriosis recurrence

Endometriosis is an oestrogen-dependent chronic inflammatory disease and an increased estrogenic effect on endometriotic tissues may aggravate the disease by genetic variations (23). The main aim of post-operative medical treatment is to suppress ovarian activity and that result in atrophy of endometriotic lesions. The success of treatment depends on the application of medical treatment after the resorption of all residual visible lesions and the eradication of the microscopic implants so that

Table 1. Table shows the risk factors for recurrence with common sides

Author, year	Risk factors
Fedele, 2004	Younger age
Vignali, 2005	
Vercellini, 2006	
Liu, 2007	
Ghezzi, 2001	Laterality of lesions
Jones and Sutton, 2002	
Waller and Shaw, 1993	rAFS stage
Busacca, 1999	rAFS>70
Parazzini, 2005	rAFS score
Abbott, 2003	
Li, 2005	
Kikuchi, 2006	
Liu, 2007	
Saleh and Tulandi, 1999	Size of cyst
Koga, 2006	
Renner, 2010	High preoperative pain
Bulletti, 2001	Absence of pregnancy
Fedele, 2004	
Li, 2005	
Koga, 2006	Previous medical treatment
Liu, 2007	
Vignali, 2005	Completeness of the first surgery
Fedele, 200	Extend of surgical excision
Li, 2005	Painful nodule

the recurrence of the disease could be minimised and the pain free period could be maintained (24, 25).

Non-steroidal anti inflammatory drugs (NSAIDs) may decrease the severity of pain, but it is difficult to say whether they have a role on recurrence rates in comparison to placebo (26). In a review by Cochrane, NSAIDs were used for pain in endometriosis, but the existing data are inadequate to say that they are effective for the treatment of pain caused by endometriosis and there is no significant difference in pain relief between types of NSAIDs (26).

Telimaa et al. (27) described that medroxyprogesterone acetate and danazol reduce pelvic pain scores more effectively than placebo when used postoperatively, but this treatment has more valuable results when used for at least six months. Morgante et al. (6) reported that low-dose danazol (100 mg/d for six months) and GnRH agonist treatment for six months postoperatively reduce pelvic pain incidence when compared with women not taking a therapy. Despite these findings, Bianchi et al. stated that there was not a significant role of danazol on postoperative recurrence rates when 600 mg/day was used for 3 months, and found no difference on recurrence rates between two groups taking danazol or not, with a ratio of 26% and 34%, respectively (28). The difference between the two studies might be related to the duration of treatment.

GnRH agonists are also used in the treatment of endometriosis after surgery (29). When compared with non users and placebo, the administration of GnRH agonists for 6 months after surgery reduces pain significantly at the 12 and 24 month follow-ups (30). Despite that study, the usage of GnRH agonists for 3 months had no effect on the pain free period in stage III/IV endometriosis (31). Jee et al. (32) reported that although postoperative GnRH agonist treatment did not reduce the objective disease recurrence in stage III/IV disease, the use of GnRH agonists delays the time to recurrence.

Oral contraceptive pills (OCP) have an important role on both recurrence rates and pain relief by the theory of ovarian inactivation, inhibition of proliferation on endometriotic lesions and also by decreasing retrograde menstruation (33, 34). Muzii et al. (34) evaluated patients who were taking low dose cyclic oral contraceptives compared to non-users after laparoscopic ovarian endometrioma excision and reported a prospective randomised controlled trial (RCT); results showed that recurrence rates were lower for the group who were using low dose cyclic oral contraceptives at the end of first year, but that the 24 and 36 month recurrence rates did not significantly differ between two groups. Koga et al. (18) also stated that the long-term recurrence rates did not significantly change in response to post-operative medical therapy.

Vercellini et al. (35) compared two groups: continuous OCP users post-operatively and those who had never used OCP; they found that continuous OCP users had a significantly reduced risk of recurrence which depended on the length of usage. There may be differences between the continuous and cyclic usage of OCP, especially from the theory that cyclic usage causes intermittent menstrual flow, which enforces the endometriotic lesions (36). Seracchioli et al. (33) reported that continuous or cyclic usage of OCP produced significantly better outcomes for recurrence when compared with the non-user group; however, there was no statistical difference between continuous and cyclic users. The continuous OCP usage might be initiated instead of cyclic treatment in the first step or scheduled if cyclic treatment fails to provide pain relief (37).

Levonorgestrel causes endometrial glandular atrophy, down-regulation of endometrial cell proliferation, an increase in apoptosis and an anti-inflammatory and immunomodulatory effect. This causes amenorrhea in most patients, and also leads to a relief in pain related to the menstrual period (38). In 2003, Vercellini et al. (39) performed a randomised controlled trial, and reported that the insertion of a levonorgestrel-*in utero* device (LNG-IUD) reduces the recurrence risk after one year when compared with patients who are expectantly observed after surgery for endometriosis. Bayoglu et al. (40) compared LNG-IUD and GnRH agonists in a prospective randomised controlled study and found no significant difference regarding chronic pelvic pain associated with endometriosis after the short and medium duration of follow-up.

From this point of view, it is important to choose the treatment according to the side effects of the administered treatment and the patient's characteristics.

Causes of recurrence

There are several studies that aim to investigate the cause of recurrence in endometriosis. The analytical determinants suggest that some subgroups with similar characteristics have high recurrence rates (14). Liu et al. (14) reported that the rate for disease recurrence is constant approximately for the first 30 months after surgery; however, the risk increases dramatically after that period, which highlights the importance of the length of follow-up period in the reported studies.

Mainly, the recurrent lesions might originate from residual lesions or alternatively from *de novo* cells coming through retrograde bleeding. Several studies demonstrate that the recurrence risk increases if the lesions are not completely removed during the initial surgery and tend to arise in the same location (9, 10). Of note, laparatomy versus laparoscopy shows comparable performance regarding the recurrence of dysmenorrhoea and pelvic pain; however, dyspareunia was higher in the laparatomy group and in those patients needing a new operation (41). The pregnancy rate also did not show a significant difference between two groups at the end of 24 months (41). For the treatment of endometrioma, excisional surgery for endometrioma was shown to be better than drainage and ablation, both for the recurrence of endometrioma, pain symptoms and fertility outcome (42). Of interest, Carmona et al. (43) reported that laser ablation resulted in higher and earlier recurrence rates when compared with endometrioma cystectomy. Fedele et al. (44) reported that the result of recurrence after repetitive laparoscopy was similar to that after the first surgery.

Lymph node involvement and lymphovascular invasion could also be responsible for recurrence, particularly in deep infiltrating endometriosis (45). Deep endometriosis also has a higher recurrence rate, which increases with time after first-line surgery. Although the radicality of the operation improves fertility outcome and reduces recurrence rates (11), a history of prior surgery and the presence of adhesions are negative prognostic factors for recurrence (19).

Approximately 10-18% of hysterectomies are performed as a result of chronic pelvic pain (46). Some studies reported endometriosis recurrence and the need for an additional surgery even after hysterectomy and bilateral salpingo-oophorectomy. Recurrent symptoms may occur in approximately 10% of patients after definitive surgery (47). Fedele et al. (48) reported that radical hysterectomy and removing infiltrating lesions achieves a better outcome and relief in symptoms when compared with conventional hysterectomy. Shakiba et al. (49) reported that the preservation of ovaries at hysterectomy increases the risk of reoperation for endometriosis-related symptoms.

The immunological factors might also play a role on the recurrence of endometriosis. The higher existence of CD15a positive Natural Killer cells in both peritoneal fluid and peripheral blood in women with endometriosis have been previously shown (50). Of interest, even after treatment with surgery or medical treatment, they do not decrease, which might cause the maintenance of an inflammatory environment for recurrence (50). The oestrogen receptor polymorphism, oestrogen receptor alpha-Pvull, may also be related to recurrence (51). Even in heterogeneous polymorphisms, the risk of recurrence increases according to the wild type.

Biomarkers for recurrence

Knowing risk factors for recurrence may allow subgroups to be identified who are at risk of recurrence. On the other hand, considering the mechanisms related to recurrence can protect the patient from unnecessary interventions and select the special treatment modality on the basis of patient characteristics (10).

Since endometriosis is an oestrogen-dependent disease, the first candidate for a biomarker is the mechanism related to it. To recall the basic principles, oestrogen receptors (ER) exist in two forms, ER-alpha and ER-beta, which exhibit an E-binding domain and a DNA-binding domain. Luisi et al. reported that the ER-alpha gene polymorphism is more associated with recurrence, especially if Pvull ER-alpha gene homozygosity is detected (51). In accordance with that, women with the PP ER-alpha genotype have higher bone mineral density and a higher risk of undergoing premenopausal hysterectomy due to leiomyoma than other genotypes (52).

Cyclooxygenase-2 (COX-2) is a rate limiting enzyme of prostoglandin (PG) synthesis and plays a crucial role in the inflammation and proliferation of endometriotic lesions (53). It has been reported that in the peritoneal fluid of infertile women with endometriosis, the concentration of PGs are increased and abnormal levels of peritoneal PGs also cause adverse effects on fertilisation, implantation and embryonic growth (54). Overexpression of COX-2 is correlated with the intensity of dysmenorrhoea (55), and non-menstrual chronic pelvic pain in women with endometriosis (56). Yuan et al. (53) evaluated 109 patients for recurrence who had histologically confirmed endometriosis from previous surgery. COX-2 expression in endometrioma tissue samples was evaluated by immunohistochemistry and 53 patients were found to have recurrence, while the remaining 56 did not. They showed a significantly higher level of COX-2 staining in the recurrent group. In other words, over-expression of COX-2 causes more frequent recurrence, and COX-2 expression level, the previous use of medication and the presence of adhesions are three important risk factors for recurrence (53).

Nuclear factor-kappa B (NF-kB) is a dimeric transcription factor that promotes the expression of more than 150 genes involved in cellular processes (57). p50, p52, p65, c-Rel and RelB are members of the NF-kB family. These all take part in DNA binding and regulation. A variety of genes are regulated by NF-kB, like genes for immune response, inflammatory processes, cell adhesion, proliferation and apoptosis (58). *In vitro* studies have shown that TNF-alpha and IL-1 alpha stimulation induce IL-6 and leukaemia inhibitory factor (LIF) production in endometrial epithelial cells, and that treatment with NF-kB inhibitor suppresses TNF-alpha or IL-1 alpha induced IL-6 and LIF production (59). NF-kB inactivation occurs through progesterone receptors (PRs). In the endometrium, there is a dynamic challenge with NF-kB activation and PR-B expression (60). Oxidants and cytokines are inducers of NF-kB activation, which in turn causes the proliferation of endometriotic cells. Therefore, NF-kB seems to have a pivotal role on the pathogenesis of endometriosis (58). Potentially, NF-kB is a

promising biomarker to identify patients with a high risk of recurrence in endometriosis. Shen et al. (61) evaluated 109 patients who had undergone previous endometriosis surgery and had a histologically confirmed endometriosis. 53 of these patients had recurrence within 30 months of surgery, whereas the remaining 56 did not experience recurrence at least 32 months after the surgery. The patient groups were similar in age, rAFS stage, menstrual phase and other variables, except for the previous use of endometriosis-related medication. The patients' archived, formalin-fixed, paraffin-embedded tissue blocks were immunohistochemically stained for the progesterone receptor isoform B (PR-B) and nuclear factor kappa-B p65 (NF-kB p65) subunit. The immunoreactivity of NF-kB p65 subunit was significantly higher in the recurrent group, whereas PR-B immunoreactivity was significantly higher in the non-recurrent group. The correlation between staining scores of NF-KB p65 subunit and COX-2 were also highly parallel. Therefore, increased NF-KB p65 immunoreactivity in relation with the decreased PR-B immunoreactivity constitutes a good biomarker for the recurrence of ovarian endometrioma, with both sensitivity and specificity ranges showing a minimum of 80%. They eclipsed the prediction power of COX-2 after regression model (61).

Conclusion

It is difficult to completely eliminate the risk of endometriosis recurrence. Various risk factors have been suggested for recurrence in the literature and these are both patient- and surgeon-dependent. According to the risk factor and the follow-up period, the recurrence rates may also alter. Finding an ideal biomarker may clarify the recurrence process, so that an individualised treatment can be maintained.

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Pregnancy of unknown location

Yeri bilinmeyen gebelik

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Abstract

Pregnancy of unknown location (PUL) is defined as the situation when the pregnancy test is positive but there are no signs of intrauterine pregnancy or an extrauterine pregnancy via transvaginal ultrasonography. It is not always possible to determine the location of the pregnancy in cases of PUL. The reported rate of PUL among women attending early pregnancy units varies between 5 and 42% in the literature and the frequency of PUL incidents has increased with the increase in the number of early pregnancy units. The management of PUL seems to be highly crucial in obstetrics clinics. Therefore, in the current review, issues identified from the literature related to pregnancy of unknown location, potential tools for prediction and algorithms will be discussed.

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Özet

Yeri bilinmeyen gebelik, gebelik testinin pozitif olduğu ancak transvajinal ultrasonografide intrauterin veya ekstrauterin gebelik bulgusunun olmadığı durumlar olarak tanımlanır. Yeri bilinmeyen gebelik vakalarında gebeliğin nihai yerini belirlemek her zaman mümkün olmamaktadır. Literatürde, erken gebelik ünitelerine başvuran kadınlarda yeri bilinmeyen gebelik hızı %5-42 arasında değişmektedir ve erken gebelik ünitelerinin sayısının artması ile yeri bilinmeyen gebelik vakalarının sıklığı da artmaktadır. Obstetri pratiğinde yeri bilinmeyen gebeliklerin yönetimi oldukça önemli görünmektedir. Bu nedenle bu derlemede, yeri bilinmeyen gebelik ile ilişkili literatür, sonuçların öngürülmesinde kullanılabilecek araçlar, ve yönetim algoritması tartışılmıştır.

(J Turkish-German Gynecol Assoc 2013; 14: 104-8)

Anahtar kelimeler: Yeri bilinmeyen gebelik, ektopik gebelik, spontan abortus

Geliş Tarihi: 23 Ocak 2013 **Kabul Tarihi:** 28 Mart 2013

Introduction

Pregnancy of unknown location (PUL) is defined as the situation when the pregnancy test is positive but there are no signs of intrauterine pregnancy or an extrauterine pregnancy via transvaginal ultrasonography (TVUS). It should be noted that PUL does not mean an ectopic pregnancy (EP). It is not a final diagnosis and in a certain number of women the final diagnosis cannot be made. In general, sonographic evaluation in addition to interval serum human chorionic gonadotropin (hCG) measurements is essential for determination of the location of pregnancy. Surgical intervention such as uterine evacuation and laparoscopy are also used in daily practice to determine or confirm the location of pregnancy. However, it is not always possible to determine the location of the pregnancy in cases of PUL since both miscarriage and ectopic pregnancy may resolve spontaneously without any treatment (1-3).

The reported rate of PUL among women attending early pregnancy units varies between 5 and 42% in the literature depending on the presence of experienced physicians and/or equipment (4). The PUL rate will probably decrease and the location of pregnancies can be diagnosed more correctly with the availability of higher resolution ultrasonography equipment (5). In this context, it is suggested to keep the PUL rate under 15% by using high resolution ultrasonography equipment and employing experienced physicians in modern pregnancy units (6).

There are two main concerns related to the management of PUL. First of all, if the pregnancy is ectopic, being diagnosed late may cause serious effects. As might be accepted, ectopic pregnancy is the most feared outcome of the PUL among all possible outcomes. Late diagnosis might increase mortality and morbidity, the success of medical treatment will decrease and more frequently a surgical intervention will be required, which may further negatively affect fertility in the following years (7). On the other side, the second concern is the overtreatment of a PUL which might potentially turn into a viable intrauterine pregnancy in the following days. Therefore, management of PUL seems to be highly crucial in obstetrics clinics.

In the current review, issues identified from the literature related to PUL, potential tools for prediction and algorithms will be discussed.

Signs of Intrauterine and Extrauterine Pregnancy in Ultrasonography

The clinicians must be aware of the indicators of not only an intrauterine pregnancy but also extrauterine pregnancy, to accurately diagnose PUL. For a normal viable intrauterine

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pregnancy, a gestational sac should be visualised under ultrasonography after a hCG level of 1500 mIU/L is noted. When the transabdominal approach is preferred, the threshold for hCG is up to 6500 mIU/L. The remarks related to intrauterine and EP should be seen in Table 1. Regarding the diagnostic importance of an EP, a non-homogeneous adnexal mass should be evaluated carefully.

The accuracy of EP might be as high as 90% (2). In some cases, there may be a collection of fluid within the endometrial cavity, which is often called a pseudosac; this has been reported in up to 20% of cases. It is not difficult to separate the pseudosac from early intrauterine gestational sac by using TVUS. A differentiating feature is the location of the fluid. An early intrauterine sac is intradecidual and is therefore seen as an eccentrically placed hyperechoic ring within the endometrial cavity, whereas a pseudosac develops within the uterine cavity and lacks a well-defined rim of surrounding echoes (8).

Conflicting results in the Literature

Although a huge number of studies have focused on the management of PUL, there is no clear algorithm for the management and predictor covering all kinds of potential results that will arise. The main reasons for inconclusive results can be summarised with the following factors: 1) The definitions of intrauterine pregnancy, miscarriage and ectopic pregnancy show wide variation among the studies. The final outcomes of women with a PUL in the literature originating from the United States have been categorised into three groups: Intrauterine pregnancy, ectopic pregnancy, and miscarriage/spontaneous abortion. The literature from the United Kingdom and European countries have been categorised by final outcomes into four groups: Intrauterine pregnancy, ectopic pregnancy, failed PUL and persisting PUL. 2) Different types of protocols are investigated for the management of PUL, and there is no comparative study. 3) The primary objective of the studies is different. Whereas ectopic pregnancy is considered the primary objective for prediction in some studies, the remaining considered abortion or intrauterine viable pregnancy as the outcome. In order to ensure homogeneity of studies, Banhart et al. (1) suggested categorising the patients into 5 groups based on their ultrasonographic findings:

- **Definite ectopic pregnancy:** extrauterine gestational sac with yolk sac and/or embryo with or without cardiac activity.
- **Probable ectopic pregnancy:** non-homogeneous adnexal mass.
- **PUL:** no finding for intrauterine pregnancy or ectopic pregnancy.
- **Probable intrauterine pregnancy:** intrauterine echogenic sac-like structure.
- **Definite intrauterine pregnancy:** intrauterine gestational sac with yolk sac and/or embryo with or without cardiac activity.

Initially, the patients might be stratified into one of those five groups based on their ultrasonography findings; however, they may be assigned to a different group during their follow-ups. The patient's symptoms and risk factors may also change the initial group of diagnosis (1-2).

Table 1. The remarks related with intrauterine and ectopic pregnancy

Ultrasound findings in intrauterine pregnancy	
Gestational sac	4.5-5 weeks
Yolk sac	5 weeks
Cardiac activity	5.5 to 6 weeks
Ultrasound findings in ectopic pregnancy	
Pseudosac	20%
Free pelvic fluid	56%
Non-homogeneous adnexal mass	60%
Gestational sac, embryo, foetal heart rate	20%

The most common final outcome among women diagnosed with PUL is the spontaneous resolution of pregnancy without the need for treatment (50-70%) (9). Those patients are considered to have either suffered a spontaneous abortion or a resorbed ectopic pregnancy. It is not always possible to determine the actual location of the pregnancy in patients with spontaneously failing PUL. Ectopic pregnancy, apparent either clinically or with TVUS, occurs only in 7-20% of the patients (5). In the remainder, clinical pregnancy becomes apparent, either viable or not (10). Only a minority of women (0.1%) will have a persisting PUL, defined as an inconclusive TVUS in combination with a slight rise or plateau in serial serum hCG levels (3).

Approaches to Predict the Outcome of PUL

There are a variety of ways to predict the outcome of PUL. Those approaches might be classified as single hCG determination, hCG ratio at 0/48 hours, single progesterone level, mathematical models with regression and lastly a combination of biochemical markers with other interventions. It is shown that the worldwide use of transvaginal ultrasonography and monitoring of hCG levels enable the follow-up of pregnancies with unknown location to be successful. Various algorithms including the combination of clinical examination, hCG follow-ups and recurring ultrasonography examinations generally succeed in determining the final location of the pregnancy in patients diagnosed with PUL. Besides these methods, measuring endometrial thickness, endometrial biopsy and cytology, inhibin A and inhibin pro-α C-RI insulin-like growth factor-binding protein 1 (IGFBP-1) are other methods that can be used to determine the location of the pregnancy. In the current literature, there is no optimal strategy to predict the possible outcome in patients diagnosed with PUL and there has been no study comparing any two of those methods for the prediction of any outcome. However, monitoring hCG levels and ultrasonographic examinations seem to be the most practical and valuable clinical tools (3).

1. Single determination of serum hCG measurement

HCG is the most commonly used hormone for the management of pregnancies with unknown location. Both single serum hCG measurements and series of serum hCG measurements can be used for predicting PUL outcomes. Single values of serum hCG to predict outcome in a PUL population is of limited value. The

reason for this is that many ectopic pregnancies in a PUL population have relatively low serum hCG levels (5). As a result of the performed meta-analysis, the sensitivity of a single hCG level for EP is found to be 11-90% and the specificity is 16-98% (3).

2. hCG ratio

The changes in serum hCG levels over 48 hour have been defined as the hCG ratio. A serum hCG increase over 48 hours of more than 66% (the hCG ratio >1.66) is a good predictor of an intrauterine pregnancy. A decrease in hCG of >13% or a hCG ratio of <0.87 has been found to have a sensitivity of 92.7% and a specificity of 96.7% for the prediction of a failing PUL; these patients have only a minimal need of subsequent follow-up (5). The sensitivity of hCG level for EP is found to be 85-100% and specificity is 28-97%. Since the study performed by Bignardi et al. (10) is the largest study of hCG levels to date, we would like to provide more detailed information from this study. Bignardi et al. (10) looked at hCG ratios with respect to the distribution of PUL results in their study. In total, 89.3% of the patients were diagnosed with ectopic pregnancy when the hCG ratio was ≤0.87, and 69.9% of the patients were diagnosed with ectopic pregnancy when the hCG ratio was between 0.87 and 1.66. When the hCG ratio was in the range of 1.66-2, the majority of patients were diagnosed with intrauterine pregnancy (56%); 39.7% of those women were diagnosed with non-viable intrauterine pregnancy and 16.3% were diagnosed with viable intrauterine pregnancy. Most of the patients (77.2%) were diagnosed with viable intrauterine pregnancy when the hCG ratio was ≥2. However, the risk of ectopic pregnancy still remains, even when the hCG ratio is ≥2, as shown by 8.2% of those patients being diagnosed with ectopic pregnancy. The hCG ratio was greater in viable intrauterine pregnancy compared with non-viable intrauterine pregnancy (3-10). According to this study, there may not be a need for further investigation and follow-up if the hCG ratio is ≥2 and if the patient does not have clinical symptoms like vaginal bleeding and groin pain. This potentially reduces the need for repeat ultrasound scans to determine viability. In women with PUL, diagnostic strategies using serum hCG ratios have the best diagnostic performance in the case of ectopic pregnancy (3).

Serum hCG levels in early viable intrauterine pregnancy

Barhart et al. (11) studied the hCG serum levels in patients diagnosed with PUL and the final outcome of viable intrauterine pregnancy. In their study, the median hCG level at the time of application was reported as 388 and the average increase in the hCG levels was reported as 50% for the first day and 124% for the second day.

Serum hCG levels in ectopic pregnancy

The rate of change in serial hCG values can be used to distinguish ectopic pregnancy from an intrauterine pregnancy or spontaneous abortion in only 73% of cases. There is no single pattern of hCG that is able to characterise ectopic pregnancy. In women with an ectopic pregnancy, 60% initially exhibited an increase in hCG values; however, this increase is lower when compared with the viable intrauterine pregnancy. In 39% of patients, the hCG values initially drop with a median slope of 15%, which is less than the mean 70%-75% decrease for complete spontaneous abortion. In 29% of patients, the hCG levels are inconsistent with increasing and decreasing hCG levels; the risk of late diagnosis and rupture is higher in these patients (12). Therefore, the increasing or decreasing hCG levels should not bias the physicians towards spontaneous abortion or intrauterine pregnancy. In women whose hCG levels are decreasing, serial hCG measurements should be performed until hCG is no longer detectable in the serum; sometimes this may take up to 6 weeks. In women whose hCG levels are increasing, ultrasonographic examination should be performed when the levels rise above the discriminatory value (2). The normal increase in serum hCG values does not exclude the possibility of a miscarriage or ectopic pregnancy (13). In fact, in 21% of women with ectopic pregnancy, the hCG levels imitate intrauterine pregnancy levels and in 8% can imitate the spontaneous abortion hCG levels. Similarly, in 1% of women with intrauterine pregnancy and in 10% of women with spontaneous abortion, the hCG levels are similar to ectopic pregnancy hCG levels (2).

Serum hCG levels in spontaneous abortion

Spontaneous abortion is the most common complication of early pregnancy and occurs in 15 to 20% of all pregnancies (2). Miscarriage defines women with spontaneously regressed serum hCG levels or women who underwent dilation and curettage with either histological features of chorionic villi, or no chorionic villi, on the condition that the postoperative serum hCG might be regressed (1). The rate of hCG clearance is dependent on the initial concentration of hCG. If the level of the first serum hCG level is high, it would decline more rapidly. The time required for hCG levels to clear following dilation and curettage is generally between 12 and 16 days (11). When a spontaneously resolving pregnancy has serum hCG levels declining to undetectable levels without surgical or medical intervention, this is defined as a failed PUL. Such a pregnancy can either be a failed intrauterine pregnancy or a resolved ectopic pregnancy; the location of the pregnancy remains undetermined (3).

3. Serum progesterone

In patients diagnosed with PUL, the serum progesterone measurement during the initial visit helps to evaluate the risk of early pregnancy complications. In this way, the number of follow-up visits can be reduced for patients diagnosed with PUL. It is not recommended to routinely follow-up patients if the serum progesterone level is ≤10 nmol/L (4). If the progesterone level is less than 20mmol/L, this is highly predictive of a failing pregnancy, whereas a progesterone level above 60 nmol/L is strongly associated with a viable pregnancy. Progesterone levels are good predictors for viability of pregnancies; however, they are not very reliable for determining the location of the pregnancy (5). High progesterone levels can be observed in both intrauterine and extrauterine pregnancies; it reflects the functionally normal corpus luteum and trophoblast. Serum progesterone is the best single marker and progesterone and hCG levels are the best way of predicting a spontaneously resolving PUL (14).

4. Mathematical models

Different mathematical models are proposed for predicting PUL outcomes. However, the accuracy of those models is not proven in different populations (3). Applying mathematical models may require complicated computations and should be verified by multiple centres.

5. Other Biochemical Markers

Inhibin A is the second most useful marker for identifying spontaneously resolving PUL after progesterone. Inhibin A levels for spontaneously resolving PUL are significantly lower when compared to the levels seen in intrauterine pregnancy or ectopic pregnancies. All pregnancies for which the inhibin A level is less than 11 pmol/L are considered spontaneously resolving PULs (14).

IGFBP-1 levels for spontaneously resolving PUL have a tendency to be higher when compared to other outcomes. The high level of IGFBP-1 reflects the defective implantation and is correlated with the increased risk of spontaneous expulsion of conception.

Inhibin pro-aC-RI levels have not found to be helpful in differentiating spontaneously resolving PULs (14).

Creatine kinase (CK) is a non-specific marker used for identifying smooth muscle injuries. CK and CK-MB levels do not contribute to distinguishing the early intra- or extra-uterine pregnancies (15).

Increase in the endometrial thickness is used to predict the normal intrauterine pregnancy in patients who are diagnosed with PUL and have vaginal bleeding. The chances for normal intrauterine pregnancy are low when the endometrial thickness is below 8mm (16).

Cancer antigen 125 (CA 125) is released from decidual cells as a result of the chorionic invasion (5). Serum CA 125 levels cannot be used to predict the outcome of PUL (17).

These novel biochemical markers are not clinically useful in predicting PUL outcomes.

6. Histological and cytological techniques in PUL

When the pregnancy location cannot be determined, the diagnostic uterine curettage and pathological examination of specimens can be used. In PUL cases, the cytology can also be used for differentiating ectopic pregnancy and spontaneous abortion (18). Uterine curettage is not recommended as routine in PUL management; however, it can be used if the potential viable intrauterine pregnancy diagnosis is excluded (5).

Management of PUL

There is no definitive management algorithm for patients diagnosed with PUL. The objective is not to diagnose ectopic pregnancy late and not to end an early viable pregnancy mistakenly. The expectant approach based on hCG and progesterone levels is a reliable method and has high success rates:

1. If the serum progesterone is <20ng/mL and Serum β-hCG is >25 IU/L, then the possible diagnosis is resolving pregnancy and a urine pregnancy test should be performed in 7 days.
2. If the serum progesterone is between 20-60 ng/mL and Serum β-hCG is >25 IU/L (<1000 IU/L), then there is a high risk of ectopic pregnancy and serum hCG should be checked within 2 days.
3. If the serum progesterone is >60 ng/mL and Serum β-hCG is <1000 IU/L, then the possible diagnosis is normal pregnancy and the scan should be repeated when hCG levels go above 1000 IU/L.
4. If the serum progesterone is >20ng/mL and Serum β-hCG is >1000 IU/L, then the possible diagnosis is ectopic pregnancy and the scan should be repeated as soon as possible or laparoscopy should be performed (14).

The majority of PULs will resolve spontaneously regardless of their location. Therefore, it is important in PUL management to focus on identifying the high risk patients by reducing the number of follow-ups and interventions (4). There are two main strategies suggested for the management of PULs:

1. Serial hCG measurements within 48 hour intervals and triaging patients due to alterations in hCG levels.
2. Measurement of the serum progesterone level during the first visit. The advantage of the clinic management protocol based on the serum progesterone level measurement is that 40% of patients can be discharged from the routine follow-up after the initial visit. This protocol reduces follow-up visits and the number of required blood tests significantly (4).

The surgical interventions performed for the diagnosis of PUL outcomes are uterine curettage and diagnostic laparoscopy. However, these surgical methods should not be routine for the management of PUL (5).

Conclusion

In summary, the frequency of PUL incidents has increased with the increase in the number of early pregnancy units. However, the PUL frequency should be kept under 15% by employing experienced doctors on this topic and using high quality ultrasonography in early pregnancy units. Several hormones have been evaluated in the prediction of PUL outcome. Among those, serum hCG level is the most useful hormone; however, evaluating the changes in hCG serum levels within 48 hours is a more reliable method than a single measurement of the serum level. However, hormone levels are very similar in the case of intrauterine pregnancy, ectopic pregnancy and spontaneous abortion. Therefore, it is important to follow-up the patients diagnosed with PUL until the final diagnosis is concluded. Even though the hCG ratio is the best method for predicting ectopic pregnancy in patients with PUL, progesterone is the best indicator for viability. Mathematical models should be verified by multiple research centres before being used routinely in clinics. There are no published randomised controlled trials that compare different diagnostic strategies for ectopic pregnancies among women who were diagnosed with PUL. There is a clinical heterogeneity between the studied populations. Therefore, there is an increasing need for studies which use a common definition for intrauterine pregnancy. There are no conclusive management algorithms for women diagnosed with PUL. However, an expectant management is appropriate for patients who are haemodynamically stable.

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Spontaneous intraamniotic hemorrhage in the second trimester mimicking an abdominal wall defect

Karın duvarı defektini taklit eden ikinci trimester spontan intraamniotik kanama

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Abstract

We report here a case of spontaneous intraamniotic haemorrhage in the second trimester which mimicked an abdominal wall defect. The ultrasound and magnetic resonance imaging findings are discussed and a review of the literature regarding differential diagnosis of bleeding and abdominal wall defects is made.

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Key words: Diagnostic ultrasound and prenatal diagnosis, intraamniotic haemorrhage, medical problems in pregnancy, premature rupture of membranes

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Özet

Biz burada bir karın duvarı defektini taklit eden ikinci trimester spontan intraamniotik kanama olgusunu sunmaktayız. Ultrason ve manyetik rezonans görüntüleme bulguları tartışılmış ve kanama ve karın duvarı defekti ayırıcı tanısı ile ilgili literatür gözden geçirilmiştir.

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Anahtar kelimeler: Tanısal ultrasonografi ve prenatal tanı, intraamniotik kanama, gebelikte tıbbi problemler, erken membran rüptürü

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Introduction

Intraamniotic bleeding occurs when a haematoma affecting the intrauterine membranes dissects through the amnion and extends into the amniotic cavity. Abdominal trauma and amniocentesis are the most common aetiologies (1). Herein we report a case of spontaneous intraamniotic haemorrhage in the second trimester which mimicked an abdominal wall defect.

Case Report

A 24-year-old primigravida female was referred to our clinic at 21 weeks of gestation with the suspicion of an abdominal wall defect. The patient presented first to an outside centre ob/gyn clinic at the 18th week of pregnancy for routine antenatal control. Laboratory results were all normal except for a mild iron deficiency anaemia and high alpha-fetoprotein (AFP) levels [2.6 multiples of median (MoM)] in the triple test. Foetal biometry performed at 18 weeks was also normal. Repeat ultrasonography (US) exam in the 20th gestational week due to high AFP levels revealed a highly echogenic mass within the vicinity of the abdominal wall. The patient was then referred with the pre-diagnosis of a possible foetal abdominal wall defect, likely gastroschisis. There was no history of trauma or genetic amniocentesis. The physical examination of the patient was normal including the blood pressure and pulse readings with no remarkable antenatal or family history.

US showed an echogenic 28x33 mm partially solid and partially cystic heterogeneous mass with unclear margins within the vicinity of the foetal anterior abdominal wall superior to the foetal symphysis pubis. The mass was freely mobile in the amniotic fluid with a cauliflower-like appearance, away from the placenta and close to the umbilical cord insertion of the foetal abdominal wall (Figure 1). There were hyperechogenic fibrin strands and speckles within the amniotic fluid and oligohydramnios was remarkable. The foetal bowels were echogenic. No additional anatomic abnormalities were noted regarding the foetus and foetal biometry was within normal limits. Colour Doppler US examination of the mass revealed no vascularisation and no blood flow. No placenta previa was detected. Due to oligohydramnios, the relationship of the mass with the neighbouring organs was not clear; therefore, foetal magnetic resonance imaging (MRI) and repeat US exam after amnioinfusion was planned. In the differential diagnosis, an abdominal wall defect like gastroschisis or intraamniotic haematoma was entertained. Foetal MRI with fat suppression revealed an intact placenta which ruled out

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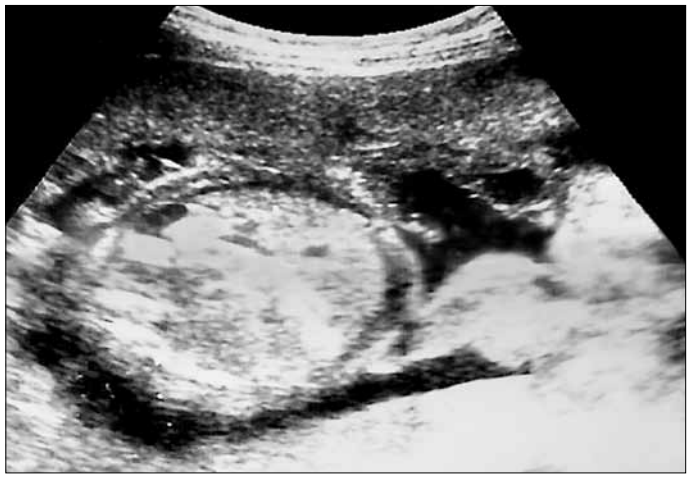

Figure 1. Grey-scale ultrasound depicts an intraamniotic highly echogenic heterogeneous mass anterior to the abdominal wall

placental abruption and an intact abdominal wall which ruled out the wall defects. The intraamniotic mass had a very dark intensity on T2-weighted images (T2WI) and was highly bright on T1WI, suggestive of an early subacute (>3 days) methaemoglobin-containing intraamniotic haematoma (Figures 2-3).

In the evening following the MRI examination, the patient had spontaneous premature rupture of membranes (PPROM) and went into premature labour with regular, painful contractions and cervical dilatation; of note, the vaginal amniotic fluid was haemorrhagic and contained blood clots. The family was informed about of the outcomes and a male baby weighing 410 g was delivered.

Postpartum cytogenetic analysis revealed a normal 46 XY karyotype and autopsy findings revealed a normal phenotype. No placental abruption was reported in the pathological examination. Based upon these findings and considering the results of clinical, imaging and pathologic examinations, a final diagnosis of spontaneous intraamniotic haemorrhage which led to PPROM and premature labour was made.

Discussion

Bleeding in the second and third trimester is a rare and a serious event with a 2% incidence and causes 25% of perinatal deaths (1, 2). Placenta previa and placental abruption are the most common causes of bleeding. The main clues to placental abruption are pain, haemodynamic deterioration and vaginal bleeding, but neither was detected in this patient (1). The patient in this case was asymptomatic; her vitals were stable throughout the event and bleeding was only noted after PPROM occurred.

Intraamniotic bleeding occurs when a haematoma dissects through the amnion and enters the amniotic cavity. Abdominal trauma and amniocentesis are the most common aetiologies (1). What predisposed this patient to an intraamniotic bleeding is unknown. The patient denied trauma or abuse-related injuries. No adverse history or medical conditions such as hypertension, preeclampsia, blood dyscrasia, vascular malformation, drug use or abuse (e.g. aspirin, coumadin, and anti-

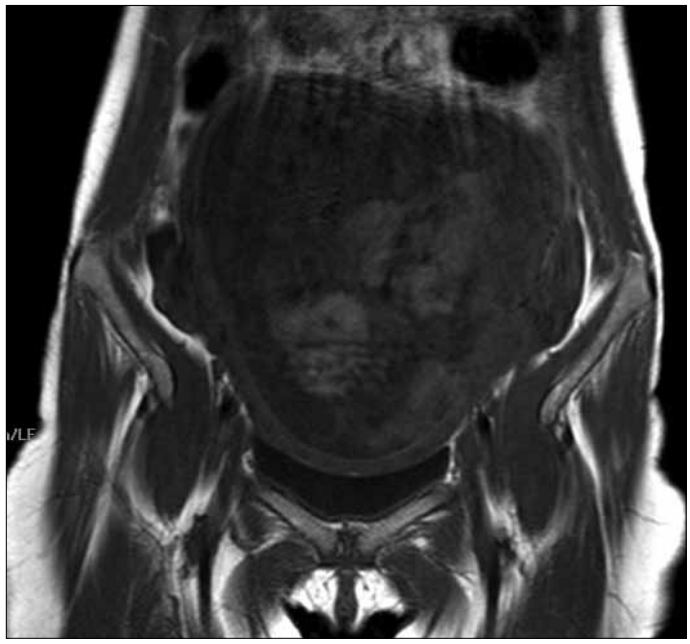

Figure 2. On coronal T1 weighted image blood products within the amniotic fluid appear as hyperintense structures against the dark background of amniotic fluid. Engulfed blood within the intestines of the fetus appears mildly hyperintense too. This finding corresponds to the "echogenic bowel" sign in ultrasound

epileptics) was present. There was no history of amniocentesis and no foetal morphologic or genetic anomaly was detected in the final pathologic examination. No placental inflammation was reported. Unfortunately, the reason for bleeding may remain unknown in 2-3% of women in the second trimester (2). Although intraamniotic haematomas have a relatively benign course with resolution from 3-10 weeks, adverse events such as preterm labour, congenital anomalies, coagulopathy, IUGR, foetal and maternal mortality and congenital anomalies are increased in case of bleeding (2, 3).

Intraamniotic bleeding, either minor or major, develops after almost every amniocentesis procedure (2). Clotted blood forms speckles, echogenic particles, complex fibrin strands or a mass-like haematoma. Fresh haematomas are usually anechoic; when organised they become echogenic and with haemolysis become anechoic again (4). Haematomas and fibrin strands may mimic anterior abdominal wall defects especially gastroschisis, amniotic band syndrome, or synechiae. The echogenic blood swallowed by the foetus may also present as "echogenic bowel" or a "gastric pseudomass" (1, 2). All of these findings were consistent with our case. The umbilical cord is an unlikely source of intraamniotic bleeding and the cause is often iatrogenic. These haematomas may also appear as anterior abdominal wall defects or placental masses because they are located especially around the umbilical cord insertion sites. Cord haematomas, however, have a grave course and most are haemodynamically instable (1).

MRI is essential in the assessment of bleeding due to its superior spatial resolution, soft tissue contrast, multiplanar imaging capacity and ability to identify and distinguish blood and

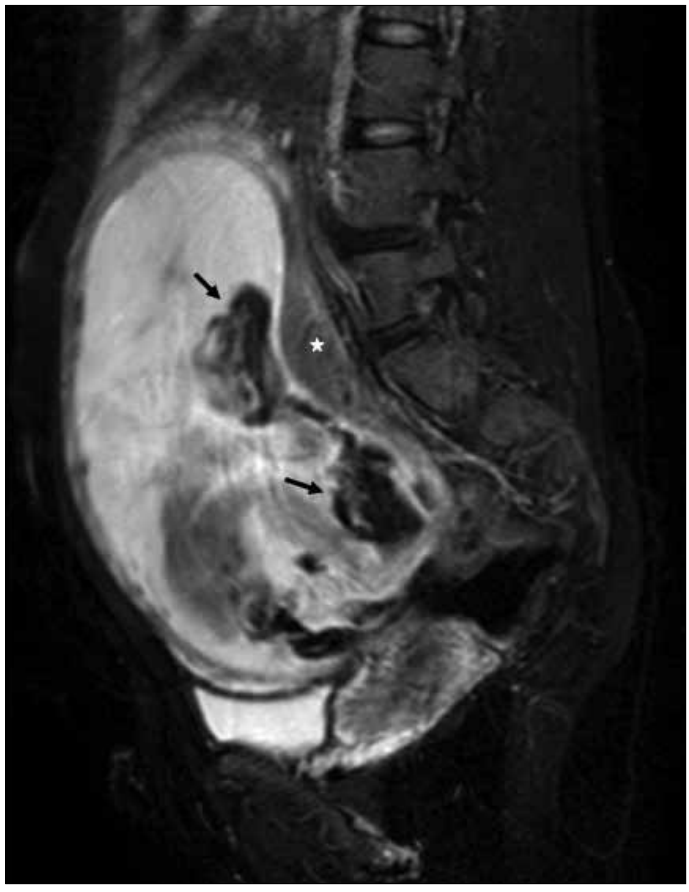

Figure 3. On this T2 weighted fat suppressed sagittal image, an intact placenta is seen on the posterior wall of the uterus (*star*). Intraamniotic blood degradation products appear as dark intensity mass-like structures in the amniotic fluid (*black arrows*).

blood products from other fluids. Diffusion WI (DWI) and T1 sequences are especially sensitive to blood. Of note, susceptibility effects of heme products are better displayed by DWI sequences. HASTE and TRUE FISP sequences are also useful when bleeding and ischemia are coexistent. Claustrophobia, high cost and availability may limit the use of MRI (2, 5).

The congenital abdominal wall defects include gastroschisis, omphalocele and body stalk anomaly (6). The ultrasonic prenatal diagnosis of these defects is relatively simple and quite possible in the first half of any pregnancy. Care should be taken not to mistake the physiological herniation of the small intestine outside the abdominal cavity between 5th and the 11th weeks of gestation as a wall defect. The sensitivity of antenatal ultrasound examination in detecting omphalocele and gastroschisis are 75% and 83% respectively (7). Misdiagnosis of omphalocele as gastroschisis occurs in 5% of patients (8). This misdiagnosis has serious implications because omphalocele is often associated with chromosomal and other severe anomalies; karyotyping is not indicated in patients with gastroschisis. In one case series, gastroschisis was misdiagnosed as omphalocele at a rate of 14.7% (9). This misdiagnosis results in unnecessary amniocentesis, which exposes the foetus to the risks and also causes psychological trauma.

There are few data in the literature about false-positive findings of abdominal wall defects in the second trimester. Walkinshaw et al. (9) described a false-positive rate of 5.3% during the mid-1980s and recently a false-positive rate of 2% was reported by Kleinrouweler et al. (10). There are some case reports which falsely presumed the presence of omphalocele in association with oligohydramnios (11-13). Diastatic and lax foetal abdominal wall with protrusion of the liver and other organs, abnormal foetal positioning with a decreased amount of amniotic fluid and failure to identify the umbilical cord have all been considered the cause (11-13). These cases are comparable to our case because the relatively small amount of amniotic fluid and compression of the abdominal wall may have caused a false positive diagnosis. Visualisation of the cord vessels and cord entry are essential in the diagnosis and differentiation of abdominal wall defects. Body stalk anomalies where the viscera is located outside the abdominal cavity in proximity with the placenta is also a mimicker, but the presence of other structural anomalies such as malformations of the heart, lungs and limbs helps with differentiation (11). Also in our case, elevated maternal AFP suggested gastroschisis clinically. High AFP is not a specific finding but is also known to increase in cases of bleeding, which may have been a diagnostic clue in this case (1, 14).

In conclusion, US has low sensitivity in cases of bleeding. Symptoms may be quite silent as well. Haematomas cause confusion in differential diagnosis and may be mistaken as other entities such as anterior wall defects. Therefore, a high index of suspicion is necessary to establish a correct diagnosis. MRI should be the imaging modality of choice in such cases.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from the patient who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept - I.Ü.; Design - I.Ü., G.B., Ş.Ş., A.F.A.; Supervision - E.S.G.G., A.F.A.;Resource - I.Ü., G.B., Ş.Ş., A.F.A.; Materials - I.Ü., G.B., Ş.Ş., A.F.A.; Data Collection&/or Processing - E.Ü., E.D.A.; Analysis&/or Interpretation - E.Ü., E.D.A.; Literature Search - I.Ü.; Writing - I.Ü., E.Ü., E.D.A.; Critical Reviews - E.S.G.G., A.F.A.*

Conflict of Interest: *No conflict of interest was declared by the authors.*

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Small cell carcinoma of the endometrium: A report of three cases

Endometriyumun küçük hücreli karsinomu: Üç vakanın sunumu

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Abstract

Small cell carcinoma (SCC) is a tumour that occurs mostly in the lung, but may be found in any organ in the body. Since SCC of the endometrium is rare, clinical behaviour and management of the disease is not well-defined. The only known prognostic factor is the stage of the disease. Here, we reported three patients with SCC of the endometrium, their management and the follow-up period.

(J Turkish-German Gynecol Assoc 2013; 14: 113-5)

Key words: Small cell carcinoma, endometrium, chemotherapy

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Özet

Küçük hücreli karsinom vücutta her organda görülebilir, ancak en sık akciğerde bulunur. Endometriyumun küçük hücreli karsinomu nadir görüldüğü için klinik davranışı ve tedavisi net tanımlanmış değildir. Bilinen tek prognostik faktör hastalığın evresidir. Bu yazıda endometriyumun küçük hücreli karsinomu olan üç hastayı, tedavisini ve takip sürecini sunduk. (J Turkish-German Gynecol Assoc 2013; 14: 113-5)

Anahtar kelimeler: Küçük hücreli karsinom, endometriyum, kemoterapi

Geliş Tarihi: 07 Ağustos 2012 **Kabul Tarihi:** 18 Eylül 2012

Introduction

Small cell carcinoma (SCC) is a tumour that occurs mostly in the lung, but may be found in any organ of the body. The cervix is the most common site in the female genital tract, whereas SCC of the endometrium is quite rare (1). To our knowledge, 80 cases of small cell carcinoma of the endometrium have been reported up to now. Since it is rare, clinical behaviour and management of the disease is not well-defined. It has a more miserable course compared to the endometrioid carcinoma of the endometrium, which is diagnosed at an advanced stage and has a poor prognosis (2). Here, we reported three patients with SCC of the endometrium and their management, one of which had an unusual course.

Case Reports

Case 1

A 52-year-old G3/P3 woman presented with postmenopausal vaginal bleeding. Her medical history was unremarkable. Pathological evaluation of her endometrial biopsy specimen revealed a poorly differentiated adenocarcinoma. Her preoperative Ca-125 was 42. During surgery, frozen section analysis of the total abdominal hysterectomy and bilateral salphingo-oophorectomy specimen was reported as an 11x6x5 cm polypoid tumour filling the entire endometrial cavity that may be uterine sarcoma or a malignant mix mullerian tumour. Therefore, bilateral pelvic and paraaortic lymph node dissection up to the left renal vein and total omentectomy were performed. Final histological examination of the specimen showed SCC of the endometrium; stage IC (International Federation of Gynaecology and Obstetrics staging [FIGO], 1988). The tumour was characterised by atypical small cells with scant cytoplasm, and some with salt and pepper chromatin configuration, forming glandular and cribriform structures (Figure 1). Immunohistochemically, the tumour cells were strongly and diffusely positive for neurone-specific enolase (NSE), strongly and focally positive for pancytokeratin and low-molecular weight keratin (LMWCK) and negative for synaptophysin, chromogranin A and S100 (Figures 2-4). She received six cycles of chemotherapy with cisplatin (80 mg/m^2, first day) and etoposide (120 mg/m^2, first three days) every 21 days. Following chemotherapy, she received external radiotherapy. Since then, the patient has been followed-up without evidence of disease for 58 months.

Case 2

A 35-year-old G1/P1 woman presented with abnormal vaginal bleeding. Her medical history was unremarkable. Pathological evaluation of her endometrial biopsy specimen revealed poorly differentiated adenocarcinoma. Her preoperative Ca-125 was 10. Total abdominal hysterectomy, bilateral salphingo-oophorectomy, bilateral pelvic and paraaortic lymph node dissection

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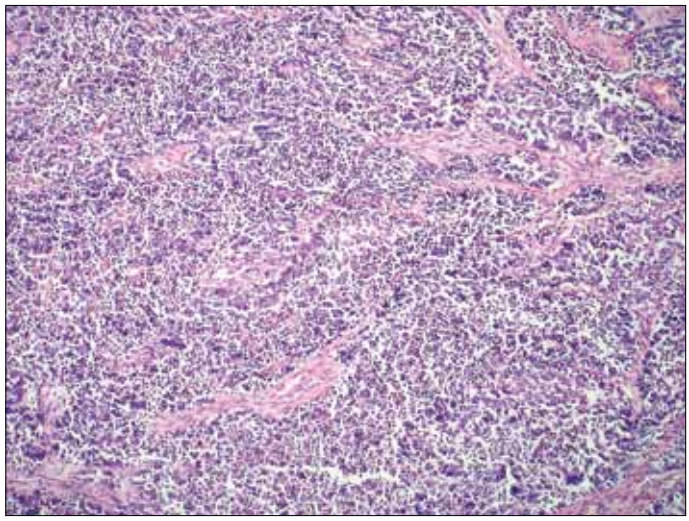

Figure 1. Small Cell Carcinoma: Atypical round small cells with scant cytoplasm and unnoticeable nucleolus; some have a salt and pepper chromatin configuration showing a diffuse growth pattern. (HEx40)

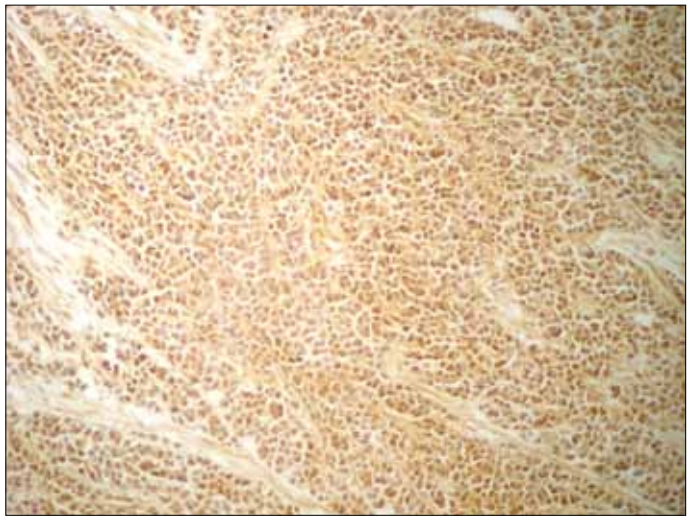

Figure 2. Small Cell Carcinoma: The tumour cells were strongly and diffusely positive for neuron-specific enolase (NSE) (x200)

up to the left renal vein and total omentectomy were performed. Final histological examination of the specimen showed undifferentiated endometrioid carcinoma of the endometrium with neuroendocrine differentiation; this was considered stage IIIC (FIGO 1988), since there was pelvic lymph node involvement. The details of immunohistochemical staining could not be obtained. She received pelvic radiotherapy and six cycles of chemotherapy with cisplatin (75 mg/m^2) and adriamycin (50 mg/m^2) following radiotherapy. She has been free of disease for 13 years.

Case 3

A 45-year-old G3/P2 woman presented with abnormal vaginal bleeding. Her medical history showed mitral stenosis, mitral insufficiency and tricuspid insufficiency. Pathological evaluation of her endometrial biopsy revealed undifferentiated carcinoma. Her preoperative Ca-125 was >500 IU/mL. During surgical exploration, massive ascites, a 100x100 mm

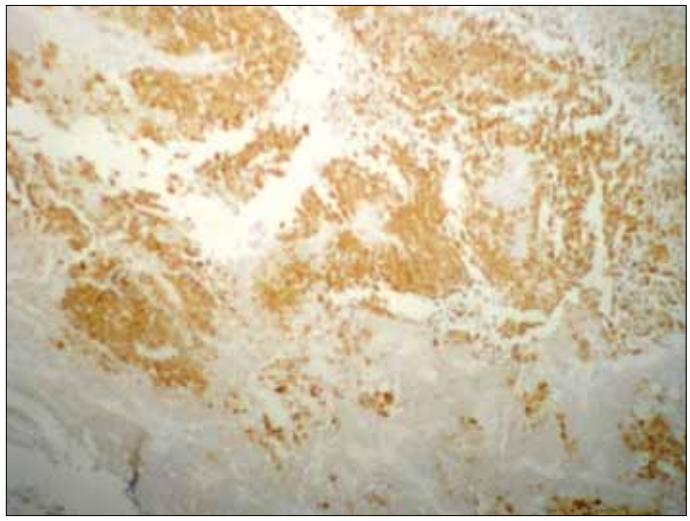

Figure 3. Small Cell Carcinoma: The tumour cells were strongly and focally positive for pancytokeratin (x100)

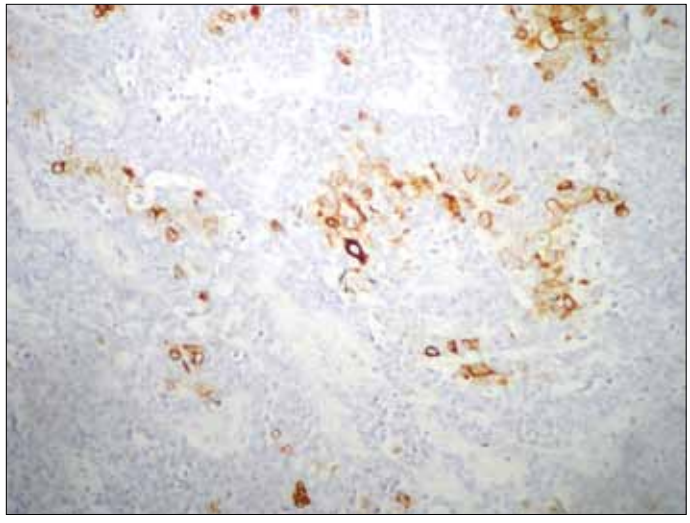

Figure 4. Small Cell Carcinoma: The tumour cells were focally positive for low-molecular weight keratin (LMWCK) (x200)

hemorrhagic omental mass that was adherent to the transverse colon, a 60x60 mm semi-solid metastatic mass in the right lower quadrant, and multiple tumoural implants up to a size of 30x30 mm on the intestinal serosal surfaces were observed. Maximal debulking was achieved by performing a type 2 hysterectomy, bilateral salphingo-oophorectomy, bilateral pelvic and paraaortic lymph node dissection up to the left renal vein, total omentectomy, and mass extraction from serosal surfaces of intestines. Pathological evaluation showed grade III endometrial carcinoma with a small cell carcinoma component, myometrial invasion, bilateral ovarian involvement, metastasis on the omentum, metastasis in the right pelvic lymph nodes and metastasis on the intestinal serosal surfaces, indicating a stage IVB endometrial carcinoma (FIGO 1988). The details of immunohistochemical staining could not be obtained. She received three cycles of chemotherapy with endoxan (1000 mg/m^2), doxorubicin (50 mg/m^2) and vincristine (1 mg/m^2). After the third cycle of chemotherapy, a 2 cm mass in the cuff

was detected upon vaginal examination. Her control abdominal tomography revealed multiple metastatic masses including spleen and liver, since her thorax tomography did not show any pathology. Concurrently, she was diagnosed with a right femoral vein embolism and underwent embolectomy. Since the disease progressed during chemotherapy, she was considered to be refractory to chemotherapy and her chemotherapy treatment was changed. She received three cycles of cisplatin (80 mg/m^2, first day) and etoposide (120 mg/m^2, first three days) every 21 days. She died of the disease seven months after surgery, following the third cycle of chemotherapy.

Discussion

SCC is a rare tumour of the endometrium with a prevalence of 1% of all endometrial carcinomas (2). The mean age of patients presenting with SCC of the endometrium is 60 years (1); however, our patients were younger. The most common complaint is abnormal vaginal bleeding (3), similar to endometrioid endometrial carcinoma. The diagnosis of SCC of the endometrium needs evidence of endometrial origin, dense sheet-like growth of morphologically similar small tumour cells and immunohistochemical staining for at least one neuroendocrine marker (4). Some of the most common neuroendocrine markers that have been reported to be positive in these tumours are NSE, synaptophysin, chromogranin A and cytokeratin (1, 5, 6). In the first case, the tumour cells were strongly and diffusely positive for NSE, strongly and focally positive for pancytokeratin and LMWCK and negative for synaptophysin, chromogranin A and S100.

Polypoid feature was suggested to be a good prognostic factor for small cell carcinoma of the endometrium in the study by Albores-Saavedra et al. (7), although the series was small. In the first case, the tumour was polypoid, but related data of the second and third cases could not be obtained.

Small cell carcinoma of the endometrium may accompany other types of endometrial carcinoma, most commonly endometrioid and adenosquamous types (2, 4, 8, 9), with similar prognoses in comparison with sole SCC of the endometrium. SCC of the endometrium may be rarely associated with paraneoplastic syndromes. These may be seen as Cushing syndrome, hypoglycaemia, visual disturbances and membranous glomerulonephritis (7), whereas a specific syndrome has not been defined peculiar to SCC of the endometrium. There were no symptoms of paraneoplastic syndrome in the three cases presented here.

Among the 80 cases of endometrial SCC that have been reported up to now, more than half of the patients with available data had stage III and IV disease (6). Two of the patients reported here had stage III and IV disease. Due to the rarity of SCC of the endometrium, management of the disease is not well-established. Actually, most of the information about the management of this cancer comes from SCC of the lung, since the behaviour of this tumour is similar. Recently, Matsumoto et al. (1) analysed the patients diagnosed up to now. What has been learned from the cases in the literature is that if diagnosed earlier, the prognosis of this cancer is better than cases with advanced disease (1). Since SCC has an aggressive growth pattern and a tendency for systemic metastasis wherever it occurs, systemic chemotherapy for metastasis and radiotherapy for local control seem logical, certainly following surgery. In terms of chemotherapy, the combination of cisplatin and etoposide has been most widely used, and it is also the preferred regimen for SCC of the lung.

The only known prognostic factor is the stage of disease. The patient in the first case had a stage IC tumour and she was free of disease for 58 months in accordance with the literature. The third case had a stage IVB tumour and died of the disease seven months after surgery. The second case does not have any evidence of disease 13 years after diagnosis, which is surprising considering that she had stage IIIC disease identified at diagnosis. More patients and more data are required to define the prognostic factors and to form a general treatment modality for SCC of the endometrium.

Ethics Committee Approval: *N/A*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept - T.T., M.F.K.; Design - I.Ü.; Supervision - G.T., N.B.; Resource - A.K.; Materials - O.L.T., H.O.; Data Collection&/or Processing O.L.T., H.O.; Analysis&/or Interpretation - G.T., N.B., I.Ü.; Literature Search - T.T., I.Ü.; Writing - I.Ü.; Critical Reviews - M.F.K.*

Conflict of Interest: *No conflict of interest was declared by the authors.*

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An unusual presentation of a submucous leiomyoma accounting to a non-puerperal uterine inversion: A case report

Submüköz myoma bağlı uterusun inversiyonu: olgu sunumu

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Abstract

Non-puerperal uterine inversion is an extremely rare gynaecological event that is usually associated with uterine tumours such as submucous or cervical leiomyomas. In this report, we describe a case of uterine inversion due to a large submucous leiomyoma in a 42-year-old multiparous and obese Caucasian woman.

(J Turkish-German Gynecol Assoc 2013; 14: 116-8)

Key words: Leiomyoma, uterus, uterine inversion, vaginal hysterectomy

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Özet

Gebelik ve lohusalıkla ilişkili olmayan uterin inversiyon oldukça nadir görülen bir jinekolojik durumdur. Genellikle submüköz veya servikal myomlar gibi uterin tümörlerin varlığı ile ilişkilidir. Bu yazıda, 42 yaşında, multipar, obez, Kafkas ırkından bir kadın hastada geniş bir submüköz myoma bağlı olarak gelişen uterin inversiyon tanımlanmaktadır.

(J Turkish-German Gynecol Assoc 2013; 14: 116-8)

Anahtar kelimeler: Myom, uterus, uterin inversiyon, vajinal histerektomi

Geliş Tarihi: 12 Haziran 2012 **Kabul Tarihi:** 14 Ekim 2012

Introduction

Uterine inversion is a rare condition. Particularly, non-puerperal uterine inversion is a much more unusual event to encounter in a lifetime for a gynaecology specialist. It emerges particularly in an over-aged group of women of reproductive age. Uterine tumours and the use of hormone replacement are the main reasons specified in the literature; particularly, the presence of increased intra-abdominal pressure was considered to promote the occurrence of uterine inversion. It was previously indicated that systemic diseases, including chronic obstructive lung diseases, cardiac diseases, connective tissue diseases, and chronic inflammatory diseases like chronic inflammatory neuropathies, play a role in such processes. Likewise, morbid obesity was acknowledged as another facilitating factor.

Case Report

A 42-year-old, obese, multiparous woman of Caucasian origin was referred to our obstetrics and gynaecology outpatient clinic, with a main complaint of heavy menstrual bleeding. She had four vaginal births, resulting in 4 healthy children. Before admission, she had a history of multiple erythrocyte suspension transfusions on several occasions because of recurrent vaginal bleeding cycles in the last 2 years. In addition, she had been experiencing intermittent lower abdominal pain for the previous nine months. She did not describe any use of alcohol or cigarettes, and no hormonal medications were being taken. Also, she did not have a prior medical history of any systemic diseases, connective tissue diseases or chronic obstructive lung diseases. Unfortunately, the body mass index (BMI) of the patient was 34.48 kg/m^2. Gynaecologic examination revealed a large, pale, regular shaped mass measuring approximately 7 cm in diameter. The mass was found to dilate the cervix, and protrude through the cervix into the vagina. With detailed bimanual pelvic examination, the mass was interpreted as a prolapsed pedunculated submucous leiomyoma with a broad base. We were not successful in vaginal ultrasonography. Similarly, abdominopelvic ultrasonography yielded inconclusive results. Vaginal bleeding was not significant at this time. She was admitted to our clinic for further evaluation and any possible blood transfusion requirements.

Complete blood count (CBC) was evaluated. CBC showed anaemia (Hb=6.7 g/dL, and Hct=20.8%) due to chronic

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blood loss with a large number of newly formed erythrocyte precursors (red cell distribution width=18.5%). Her vaginal bleeding increased moderately and descent of the mass was augmented in the first 24 hour period of her clinical follow-up. CBC was checked at 2-hour intervals. The mass totally prolapsed throughout vulvar introitus, and it became visible around the perineum upon a basic view. After a couple of hours, she showed signs of hypovolemic pre-shock comprising orthostatic hypotension and syncope following minor head raising, heavy sweating throughout the whole body, but particularly on the head and face, involuntary tremor in the extremities, and mild to moderate tachycardia and hypotension, but without apparent blood loss vaginally. At this time, Hb and Hct were measured as 5.5 g/dL and 16.1%, respectively. Transfusion with packed erythrocyte suspensions was started. At the same time, emergency surgery was planned comprising excision of the submucous leiomyoma transvaginally. Informed written consent was also taken from the patient in order to proceed for vaginal or abdominal hysterectomy. Gynaecologic examination under general anaesthesia revealed that the mass was not in fact pedunculated. The mass was fully palpated in the vagina, but no obvious demarcation line could be perceived between the mass, the apparent massive submucous leiomyoma, and the uterine cervix (Figure 1); the mass was coming out of all vaginal borders. We thought that the mass was composed of two parts encapsulating the distal and proximal parts. The leiomyoma, which was found within the vagina, composed the distal part, whereas the uterine corpus remained upwards in the pelvis. Dissection of the caudal part of the leiomyoma from its base through vaginal retraction was performed. We progressed through the procedure by dissecting, dividing and suturing the pedicles (Figure 1). After two steps of this surgical procedure, we were faced with a glossy surface on the inner layer of proximal part of the mass. We were convinced that this structure was the uterine serosa, but realised that the uterus inverted spontaneously, and that the uterus itself constituted the proximal part of the mass which was in continuation with the submucous leiomyoma with a broad base. A urethral catheter was then inserted and the procedure was performed as a conventional vaginal hysterectomy (Figure 2). The Foley catheter remained for two days and the patient was discharged without any complications.

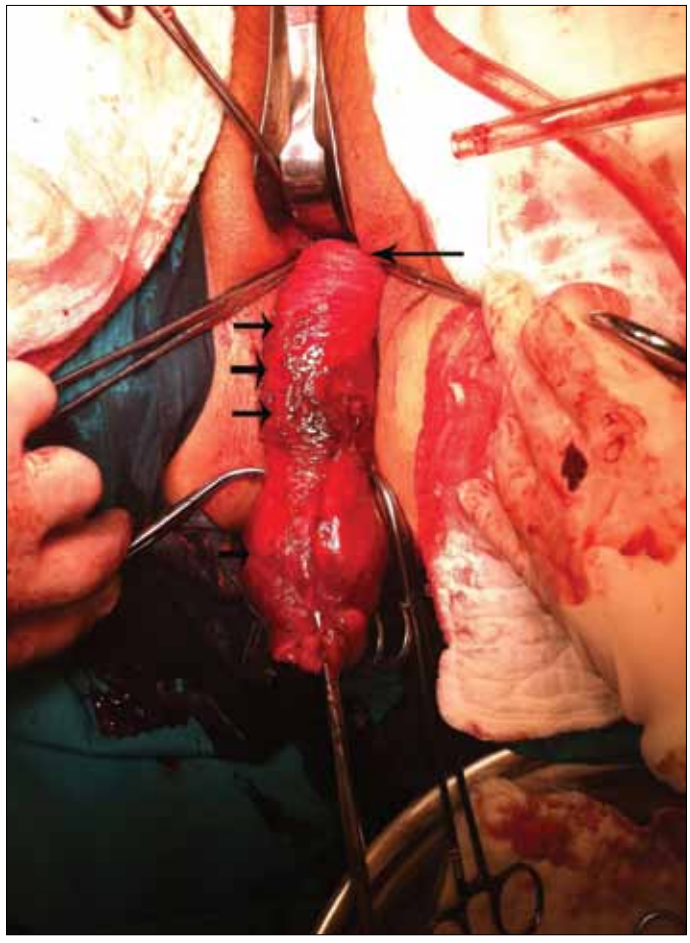

Figure 1. Intraoperative view of a hysterectomy which was performed transvaginally. The uterine serosa border is shown by a long arrow with a straight clamp at the top. Triple arrows show inverted uterine corpus. Short arrow indicates the submucous myoma, whereas curved clamps indicate the base of the tumour

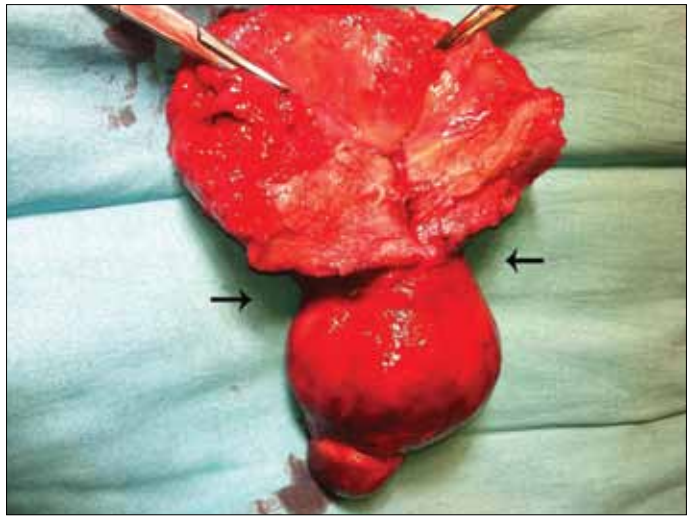

Figure 2. Postoperative picture of the submucous myoma within the uterus. Black arrows indicate the submucous myoma along with its broad base. Round ligaments which remained at the interior compartment are shown by straight clamps

Discussion

Non-puerperal inversion of the uterus is seen in extremely rare instances (1-3). The rate of misdiagnosis is higher than expected, as in our case. Mwinyoglee et al. reported in their review that 97% of the cases are associated with tumours, 20% of which were malignant (2).

The diagnostic value of transvaginal ultrasonography is limited, especially in cases with large masses protruding into the vagina. Principally, because of physical reasons one cannot administer the probe, as in this case, into a choked vagina. As another concern, ultrasound beams could not be transmitted beyond the mass, so technically one would gather poor quality sonographic images. Therefore, magnetic resonance imaging (MRI)

is the preferred diagnostic tool, and can provide an accurate diagnosis before surgery (3, 4). MRI can present the dimensions, and, more importantly, the extent of the tumour, and aids in the accurate diagnosis and treatment of the patient (4). We planned to get MRI sections in the case described; however we did not have time to perform the MRI because symptoms of vasovagal syncope and hypovolemic shock including hypotension, progressive pain and sweating emerged within the first 24-hours of the hospital stay. Non-palpation of uterine corpus on bimanual examination and non-visualisation of the uterine cervix after progressive excision of the vaginal mass as stated by Lascarides et al. gave clues for the correct diagnosis (5).

Reposition procedures, according to the reproductive desire of the patient, or hysterectomy could be considered for surgical treatment. Spinell and Kustner techniques are the two most accomplished organ-preserving vaginal approaches reported in the literature. In the Spinell procedure, an anterior longitudinal uterine incision is made after dissecting the bladder, while in the Kustner technique, the incision is performed in the posterior wall. After the incision is completed, both infundibulopelvic ligaments are clamped and ligated to prevent thromboembolic events, as suggested by Kopal et al. (6). Hysterectomy is the other alternative modality, and can be performed by both vaginal and abdominal routes. As uterine inversion is quite uncommon, most cases are misdiagnosed. In the case of a vaginally-protruding mass, uterine inversion should be kept in mind as a differential diagnosis, particularly upon detection of submucous myomas larger than 4 cm in diameter. Once identified, vaginal hysterectomy could be chosen as a safe surgical modality. One should keep in mind that it is important to take biopsies from tumours before definitive surgery is planned because there is a 20% chance of missing the diagnosis of malignant tumours of the uterus or cervix. Also, adequate surgical management requires experience in vaginal surgery.

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Informed Consent: *Written informed consent was obtained from the patient who participated in this study.*

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Prenatal diagnosis of fetal ovarian cyst: case report and review of the literature

Fetal over kistinin prenatal tanısı: olgu sunumu ve literatürün gözden geçirilmesi

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Abstract

Foetal ovarian cysts are the most frequently encountered intra-abdominal cystic masses diagnosed prenatally. The aetiology of foetal ovarian cysts is still unknown, but hormonal stimulation is generally considered to be responsible for the disease. The diagnosis is made by the exclusion of other cystic lesions confined to the foetal abdomen.

In this article we report antenatally-detected foetal ovarian cyst with a review of the available literature. Antenatal ultrasonography (USG) revealed an abdominal cystic mass 41x33 mm in diameter in a 33-week gestation female foetus. The normal anatomy of other foetal abdominal organs suggested that an ovarian cyst was the most likely diagnosis. In the antenatal follow-up period, the cyst diameter increased with time. After delivery, USG scan confirmed the antenatal findings. Due to abdominal distension and respiratory distress, ovarian cystectomy was performed on the second postnatal day. The histopathological evaluation of the surgical material reported a serous cystadenoma of the ovary with non-malignant properties.

(J Turkish-German Gynecol Assoc 2013; 14: 119-22)

Key words: Ovarian cyst, prenatal diagnosis, histological examination

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Özet

Fetal over kistleri, prenatal dönemde tanı konulan kistik abdominal kitleler arasında en sık gözüken yapıdır. Etyolojisi halen bilinmemekle birlikte, hormonal stimülasyon genellikle bu durumdan sorumlu tutulmaktadır. Fetal batın içinde yerleşen diğer kistik lezyonların dışlanmasıyla tanısı konulmaktadır.

Bu makalede antenatal saptanan fetal over kisti olgusu literatürün gözden geçirilmesiyle birlikte sunulmuştur. Otuz üç haftalık dişi bir fetusta, yapılan antenatal ultrasonografide (USG) 41x33 mm boyutunda abdominal kistik kitle saptandı. Diğer fetal abdominal yapıların normal olması, tanının over kaynaklı kist olduğunu kuvvetlendirdi.Antenatal takipler sırasında kist çapında zamanla artış saptandı .Postnatal dönemde yapılan USG, antenatal dönemdeki bulguları doğruladı. Yenidoğanda abdominal distansiyon ve solunum sıkıntısı gelişmesi üzerine ovarian kistektomi uygulandı. Histopatolojik inceleme sonucu over kistinin, malign özelliği olmayan seröz kistadenom olduğu rapor edildi. (J Turkish-German Gynecol Assoc 2013; 14: 119-22)

Anahtar kelimeler: Over kisti, prenatal tanı, histolojik inceleme

Geliş Tarihi: 11 Ağustos 2012 **Kabul Tarihi:** 14 Ekim 2012

Introduction

Foetal ovarian cysts are the most commonly diagnosed abdominal tumours in the prenatal period (1). They are usually unilateral, and diagnosed in the third trimester.

Although the aetiology is still unknown, hormonal stimulation is generally considered to be responsible for the disease (foetal gonadotropins, maternal oestrogen and placental human chorionic gonadotropin). The decrease of hormonal stimulation after delivery may lead to spontaneous resolution of the cyst. Abnormal development due to the disruption of vascularisation in primitive gonad, foetal hypothyroidism, congenital adrenal hyperplasia, mutation of the G protein α-subunit, and increased placental chorionic gonadotropin levels in complicated pregnancies such as in diabetes, pre-eclampsia and Rh isoimmunisation have been so far reported to contribute to the development of these cysts (2-6). Foetal and maternal ovarian cysts may co-exist and could potentially have a similar hormonal aetiology (7).

Renal cyst, hydronephrosis, megacystis, anorectal atresia, urachal cyst, meconium pseudocyst, hydrometrocolpos, mesenteric cyst, liver and splenic cyst may also cause cystic masses in the foetal abdomen; therefore, a differential diagnosis should be established in these circumstances.

Case Report

A primigravid 19-year-old woman was referred to our prenatal centre at 33 weeks of gestation following the ultrasonographic detection of a foetal cystic mass in the abdomen. The

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past medical history and antenatal course were unremarkable. Ultrasound examination demonstrated normal anatomy of the bladder, kidneys, liver and intestine in a female foetus. The cyst measured 41x 33 mm in diameter, had anechoic content and the thin wall was located in the lower foetal abdomen; these features suggested a simple ovarian cyst (Figure 1). The patient was informed about the condition and serial ultrasonographic scans were performed every 2 weeks until birth. Cyst diameter was increased to 55x53 mm, 75x56 mm and 82x63 mm at 35, 37 and 39 weeks of gestation, respectively. Caesarean section was performed because of breech presentation at term and a healthy female infant weighing 3040 grams with Apgar scores of 8 and 10 (after 5 and 10 minutes, respectively) was delivered. Abdominal ultrasonographic scan performed on the first postnatal day revealed a cystic mass of 85x85 mm in diameter. Due to severe abdominal distension and respiratory distress, laparotomy was recommended on the second postnatal day. During the surgical procedure, a yellow cystic structure in the right lower quadrant arising from the right ovary was seen (Figure 2); the pedicle was not twisted and the left ovary and adnexal structures were normal in appearance. Cystectomy of the right ovary was carried out. The histopathological examination revealed a large yellowish cystic mass with a diameter of 90x90 mm filled with serous fluid. Final pathological report concluded a serous cystadenoma of the right ovary (Figure 3). On the 5th day after surgery, the newborn had undergone an uncomplicated postoperative course and was discharged with her mother.

Discussion

The incidence of foetal ovarian cyst is uncertain but has been estimated at 1 in 2.625 pregnancies (8). The increased use of routine USG has led to the earlier detection of foetal ovarian cysts. The first case detected by USG was reported in 1975 (9); the earliest case was described in the 19th week of gestation (10). Most cysts are small and involute within the first few months of life.

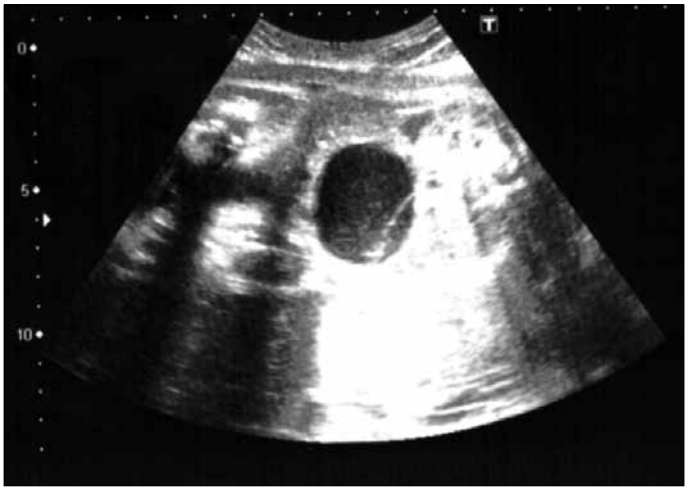

Figure 1. The ultrasound finding of ovarian cyst with a diameter of 41x33 mm at 33 weeks gestation

Magnetic resonance imaging (MRI) is able to visualise ovarian cysts *in utero* by providing exact characterisation through supplemental findings like superior soft tissue contrast, but USG represents the diagnostic standard for imaging in the perinatal period (11). Moreover, a sonographic clue for the diagnosis of foetal ovarian cysts, termed as "daughter cyst sign", was reported. Within the foetal ovarian cyst, a single, round, anechogenic structure adjacent to the cyst wall was found. This feature confirmed the ovarian origin of the cyst with a sensitivity of 82% and specificity of 100% (12).

Foetal ovarian cyst are classified according to ultrasonographic criteria into two groups: simple (uncomplicated) and complex (complicated) (11). The characteristics of ultrasonographic appearance of simple cysts are the following: unilocular, anechogenic, round, small size (often<5 cm), unilateral or seldom bilateral and thin-walled. Complex cysts on the other hand

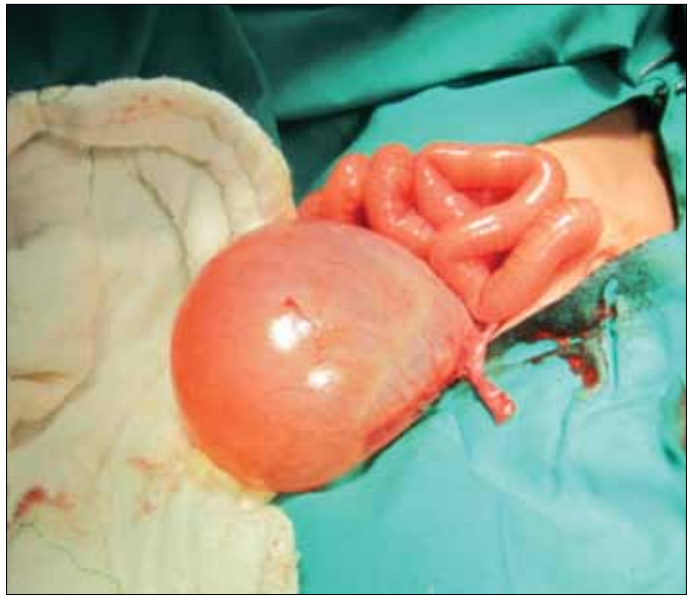

Figure 2. Intraoperative appearance of cyst

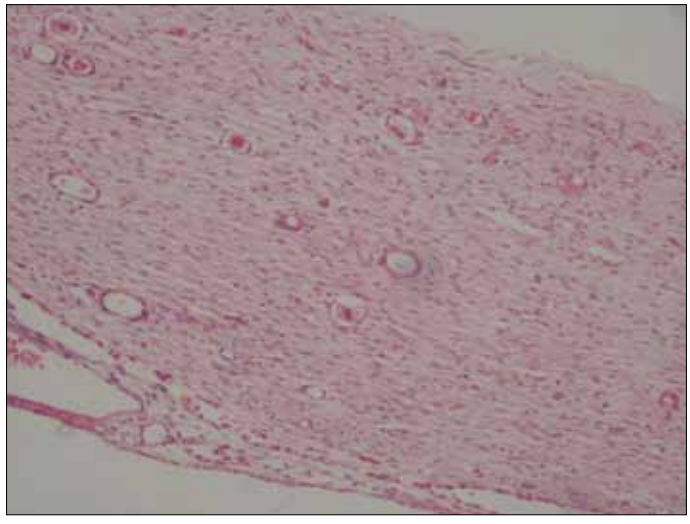

Figure 3. Light microscopy finding in the cyst wall that shows columnar epithelium (H&E, x100)

are thick-walled, containing hyperechogenic components and free-floating material with intracystic septations. Cyst diameter and echogenicity are the main criteria for establishing prognosis. Serial ultrasonographic examination to detect any structural changes in size, appearance or complications is important during the prenatal period. In accordance with the literature, our case report shows that current prenatal USG provides sufficient diagnosis of ovarian cyst.

Various complication associated with foetal ovarian cysts may occur including rupture, haemorrhage, ascites, compression of other viscera and ovarian torsion. Torsion and haemorrhage may lead to loss of the cystic gonad. Rare complications like oedema of the labia and moderate ascites in a foetus with true hermaphroditism, spontaneous haemorrhage into a foetal ovarian cyst, transient ascites, autoamputation, or polyhydramnios as a result of intestinal obstruction were also reported (13-16).

Torsion is one of the most serious complications that occur more frequently during foetal life than post-natally. It may be seen antenatally in up to 38-55% of cases and 50-78% during the neonatal period (17). Torsion may also result in adhesion of the ovary to the bowel or other pelvic organs. Sonographic findings of adnexal torsion are not specific. A possible sign of torsion is foetal tachycardia, probably because of peritoneal irritation. Haemorrhage within a cyst is usually found in association with torsion; therefore imaging methods (USG or MRI) may not able to distinguish these complications (18, 19). There are contradictory reports between size of the cyst, length of the ovarian pedicle and the risk of torsion as well as haemorrhage (1, 11, 20). Long-term outcome after perinatal ovarian torsion is unknown. Haemorrhage within a cyst is another important complication. In a review of 82 foetal ovarian cases, poor outcome linked to ultrasonographic signs of intracystic haemorrhage was stated (21).

The question of perinatal management of ovarian cysts is not clearly answered and is seldom based on the clinical status of the patient. In addition, clinical management varies widely among different centres. Prenatal or postnatal aspiration, neonatal surgery and simple USG monitoring are acceptable with the main objective of preserving ovarian parenchyma.

In most cases, simple cysts tend to regress spontaneously in a sonographic follow-up period. Conservative approaches with serial USG monitoring are recommended according to a case report series by Sanchez et al. (22). The rate of spontaneous resolution of simple cyst is higher than that of complex cysts; some investigators have recommended conservative management for all types of cysts regardless of their sonographic appearance (23, 24). In the postnatal period, no surgical intervention may be needed if the cyst regresses spontaneously. The importance of visualisation of the course of the ovarian cyst was stated in our case. Although the cyst was simple with a small size initially, a progressive increase in cyst size became more evident during the follow-up period.

The importance of aspiration of ovarian cysts exceeding a 40 mm diameter was reported by Noia et al. (25). The authors emphasised that aspiration of ovarian cysts allows a good longitudinal treatment of foetal affection, thus avoiding torsion, tissue necrosis and postnatal surgery. The suggested threshold size for prenatal aspiration of simple cysts varied from 30 to 50 mm in most reports (17, 20). Complications of large cysts include the impairment of spontaneous delivery, distension of the foetal abdomen and disruption of the foetal heart function. Prenatal puncturing is reasonable under these conditions before birth, but risks of preterm labour, chorioamnionitis, foetal injury and foetal pain are of concern (18). Recession of functional abnormalities in foetal echocardiography after prenatal aspiration of a foetal ovarian cyst was also noted (26). Another consideration of puncturing is intracystic bleeding, which can lead to subsequent diagnostic difficulties and the possibility of foetal malignant ovarian tumour (4, 10). An additional advantage of this procedure is performing a hormonal and cytological analysis of the cyst fluid, eliminating the need for laparotomy on a newborn. In some circumstances, the hole produced by aspiration closes again and the cyst reforms. There has been no published randomised trial comparing prenatal aspiration of the cysts with any kind of postnatal management.

There are controversial reports about the postnatal surgery indication. The list of major indications of surgery include: complex cysts that showed evidence of torsion post-natally, the persistence of large cyst in a follow-up period, the suspicion of neoplastic tumour and clinical symptoms in the newborn like abdominal distension, bowel obstruction or torsion. Abdominal distension and respiratory distress due to a large-sized ovarian cyst with compression of other visceral organs was determined in our case. Benefits of surgical management include the definite removal of the cyst for optimal ovarian preservation and separation of any adhesions between ovaries and pelvic organs. Dimitraki et al. (27) suggested treatment of symptomatic cysts or cysts with a diameter of >5 cm which do not regress or enlarge, whereas another report suggested neonatal surgery in the case of complex cysts regardless of size or in simple cysts larger than 20 mm in diameter (19). Surgical methods via laparoscopy or laparotomy can be performed depending on the experience of the surgeon. Two-port laparoscopy has been used in the management of foetal ovarian cyst that is cosmetically preferable and allows earlier feeding and recovery (28). Surgical intervention through a Pfannenstiel incision was carried out in the current case due to the large size of the cyst.

Ovarian cysts are most often functional and benign tumours; the question of malignancy plays virtually no role in the prenatal diagnosis. In most circumstances the origin is the follicular epithelium, but they can also occur as theca-lutein or corpus luteum cysts. Foetal ovarian carcinoma has been reported by Ziegler in a 30-week foetus (29). A review of the literature reveals that 85-90% of foetal ovarian masses are cystic (follicular or luteinic origins), and 10-15% are organic (3% carcinomas and 7-12% represented by teratomas, and mucinous and serous cystadenomas) (30).

Mode of delivery is not essentially affected by prenatal diagnosis of an ovarian cyst. Today, unless an obstetric indication is present, vaginal delivery is recommended (4, 18).

Long-term outcome of children with ovarian cysts diagnosed prenatally is limited. A higher rate of ovarian loss was noted in children whose prenatal USG showed a simple ovarian cyst that became complex in postnatal scans. Long-term pelvic

ultrasound follow-up in order to monitor the integrity of pelvic organs was recommended (31).

Finally, a foetal ovarian cyst is not a life-threatening condition. They are usually simple and small in size. After diagnosis, they must be followed by serial ultrasonographic examinations.

Ethics Committee Approval: *Ethics committee approval was received for this study.*

Informed Consent: *Written informed consent was obtained from the patient for publication of this case report and any accompanying images.*

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept - O.E.; Design - S.Ö.; Supervision - O.E.; Resource - M.K.; Materials - O.E.; Data Collection&/or Processing - B.S.İ.; Analysis&/or Interpretation - M.B.E.; Literature Search - O.E.; Writing - O.E.; Critical Reviews - O.E.*

Conflict of Interest: *No conflict of interest was declared by the authors.*

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What is your diagnosis?

A twenty-three year old woman, gravida 2, parity 1, applied to her primary gynaecologist with the complaint of chronic right lower quadrant pain. She had a history of one normal vaginal birth 4 years ago and dilatation & curettage 3 months ago for an unintended pregnancy. Her last sexual intercourse was 4 months ago. Transvaginal sonography revealed a 6 cm right adnexal mass that appeared to be an ovarian malignancy and she was referred to our clinic. Upon physical examination, she had minimal tenderness on the right lower quadrant but there were no defance or rebound. Transvaginal sonography that was performed in our department revealed a 53x62 mm thick walled cystic mass with papillary projections (Figure 1). There was minimal free fluid in the pouch of Douglas. Magnetic resonance image (MRI) supported the diagnosis of ovarian tumour with high T2 signal intensity (Figure 2). CA-125, AFP, LDH, CA-19-9 levels were within normal ranges; only β-hCG level was 38 mIU/mL.

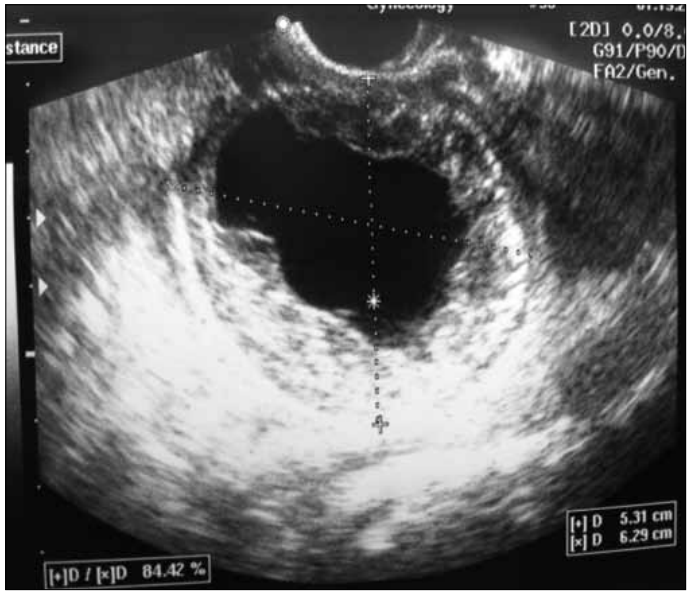

Figure 1. The sonographic view of the right adnexal mass.

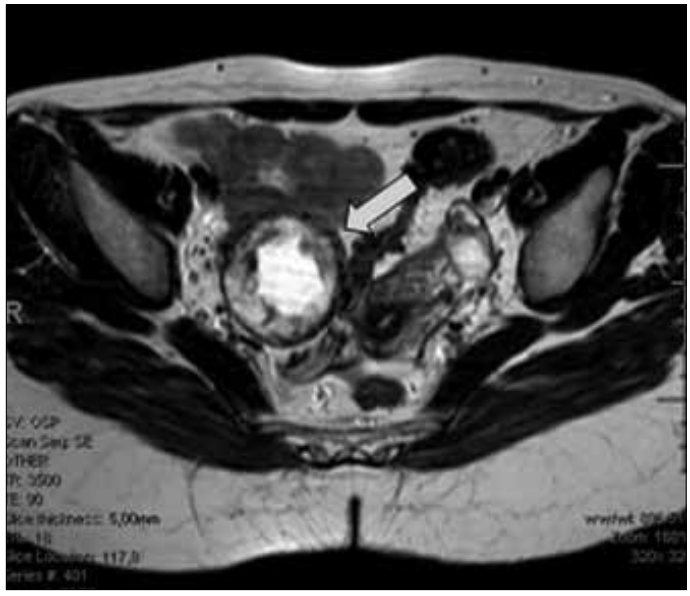

Figure 2. Arrow shows the magnetic resonance imaging of the adnexal mass.

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Answer

Chronic ectopic pregnancy is a rare type of ectopic pregnancy. Its true incidence is not really known, but in some studies it is reported to account for 6-20% of all ectopic pregnancies (1-3). It results from minor bleeding from the tubal pregnancy or abortion. The mass may turn into a haematocele that contains trophoblastic tissue. Mostly, adhesions may occur around due to the inflammatory response (2, 3). Negative >β-hCG level does not rule out chronic ectopic pregnancy because β-hCG may be negative or near normal (4). Clinical symptoms are not reliable because there is no specific symptom; abdominal pain is mostly chronic or mild. Thus, preoperative diagnosis is difficult.

The imaging techniques generally do not help to diagnose because the appearance can overlap with acute pelvic inflammatory disease, pelvic abscess, vascular tumours, and endometriosis (5). As described by Su et al. (6), the lesion may be a cystic mass with intralesional haematoma and soft tissue components, so it may be misdiagnosed as an ovarian tumour. Also the ensuing inflammatory reaction can incorporate the uterus and may make the margins of the mass indistinct (7). In our case, although other tumour markers were negative, β-hCG level was 38 mIU/mL, her last sexual intercourse was 4 months ago and her menses were regular; therefore, we did not suspect pregnancy. Both the ultrasound and the MRI showed an atypical mass that is cystic and solid and has papillary projections. In the MRI, the mass has high T2 intensity. With all of these findings, the possible preoperative diagnosis was germ cell tumour; therefore, we performed a laparotomy. During the operation, it appeared that the mass had originated from the right fallopian tube and there was no adhesion around the mass. There was minimal free fluid in the pouch of Douglas, and the gross view of the lesion did not appear malignant. Also, the frozen section supported the fact that it was benign and contained trophoblastic tissue. This case showed us that this typical sonographic view of a thick-walled cystic mass with a flower-leaf pattern around it may be an indication of a tubal pregnancy, especially if the ovary can be seen separately. Harada et al. also showed a similar image in their case (8). Additionally, the β-hCG level may help to differentiate this diagnosis if it is positive.

As a conclusion, chronic ectopic pregnancy is a rare pathology and may mimic ovarian cancer. The clinician must also consider this diagnosis if the image of the lesion is similar to our case and should also use minimally invasive treatment to reach the correct diagnosis.

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Questions on the article within the scope of CME/CPD

1. Which of the followings is not a risk factor for recurrence of endometriosis?
   a) Size of the cyst
   b) Advanced stage disease
   c) Having a history of previous endometriosis surgery
   d) Pregnancy
   e) Deep pelvic endometriosis history

2. Which of the following biomarkers could determine the recurrence of endometriosis?
   a) NF-kB
   b) Ca 125
   c) CEA
   d) Ca 19-9
   e) LDH

3. Which of the following medications could decrease the recurrence risk of endometriosis when used post-operatively?
   a) Danazol
   b) NSAID
   c) COC
   d) Medroxyprogesterone acetat
   e) IUD

4. Which of the following surgical approach has the minimum recurrence rate for deep pelvic endometriosis?
   a) Laser ablation
   b) Cystectomy
   c) Cyst drainage
   d) Coterization
   e) Excision of all the endometriotic parts with adhesiolysis

5. What is the average two-year recurrence rate for endometriosis?
   a) 19.1%
   b) 20.8%
   c) 14.6%
   d) 5%
   e) 25%

6. For which of the following one recurrence rate of endometriosis is higher than others?
   a) Umblicus
   b) Ovary
   c) Cervix
   d) Uterine wall
   e) Deep pelvic

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12-15 June 2013	**11th Annual Meeting of ISSCR** Boston, MA, USA http://www.isscr.org/home/annual-meeting
19-22 June 2013	**11th World Congress of Perinatal Medicine** Moscow, Russia http://www.wcpm2013.org/
23-27 June 2013	**12th World Congress in Fetal Medicine 2013** Marbella, Spain http://www.fetalmedicine.com/fmf/courses-congress/conferences/
24-26 June 2013	**RCOG World Congress in Obstetrics and Gynaecology 2013** Liverpool, England http://www.rcog2013.com/index.htm
7-10 July 2013	**29th Annual Meeting of ESHRE** London, England http://www.eshre.eu/ESHRE/English/Annual-meeting/Istanbul- 2012/page.aspx/1381
28-31 August 2013	**SLS - Minimally Invasive Surgery Week / Annual Meeting and Endo Expo.** Reston, VA, USA http://laparoscopy.blogs.com/ee06/
18-21 September 2013	**10th Congress of the European Society of Gynecology (ESG)** Brussel, Belgium http://www.seg2013.com/

NATIONAL MEETINGS

30 May - 2 June 2013	**The World Congress on Building Consensus out of Controversies in Gynecology, Infertility and Perinatology** İstanbul, Turkey http://www.bcgip.com/2013/Default.aspx
6 - 8 September 2013	**SGI Summit Turkey 2013: Innovations in Obstetrics and Gynecology** İstanbul, Turkey http://www.sgiturkey2013.org/
26-29 September 2013	**4th Congress of the Society of Reproductive Medicine** Antalya, Turkey http://www.utd2013.org/
26 - 29 September 2013	**5th National Osteoporosis Symposium** Muğla, Turkey http://www.osteoporoz2013.org/
8 - 10 November 2013	**Maternal Fetal Medicine and Perinatology Association of Turkey Ultrasonography Course** İstanbul, Turkey http://www.tmftpultrason2013.org/
20-23 November 2013	**3rd National – 2nd Congress of Midwifery** Antalya, Turkey http://www.ebko2013.org/
23-24 November 2013	**Derin İnfiltratif Endometriozis Sempozyumu** İstanbul, Turkey
24-26 November 2013	**6th National Urogynecology Congress** İstanbul, Turkey http://urojinekoloji2013.org/
5-6 December 2013	**8th National Congress of Menopause Osteoporosis** İstanbul, Turkey http://turkiyemenopozosteoporoz.org/