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Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Colleagues,

Our journal continues to be the most influential, significant, trusted and high-quality clinical journal of obstetrics and gynecology in our country. In order for our journal to be preferred worldwide as well as in this region, I believe that your contributions play a very important role to reach our mission. I expect full support of our colleagues by selecting and following our journal.

I especially thank the editorial board for what we have achieved so far together.

Even though being an obstetrician and gynecologist means encountering many obstacles nowadays, I believe that a well trained and educated obstetrics and gynecology physician will always have a remarkable place in our country, as well as all over the world.

I ask all our colleagues for your precious support, efforts and contributions. We encourage all our colleagues for making new researches and publications, creating papers; since it would be an honor for us to help them develop in expanding their academic obstetrics and gynecology research skills.

Consequently, since we can develop further together, I kindly request that all colleagues would always be sharing within and supporting each other.

Sincerely,

Ateş Karateke, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

Our journal has two reviews, nine original articles and two case reports. I believe that they are detailed and original contributions fort he obstetrics and gynecology literature. I would like to thank to the contributors for sharing their knowledge and findings with us.

Also we begin to publish and wellcome research on sexual medicine and cosmetic gynecological surgery both of which are taboo in our country. Although some clearly oppose genial aesthetic procedures increasing demand also necessitate clear evidence. Long term results and complications of the proposed procedures on the female genitalia.

Our society and journal is also suporting the research on improving resident and postgraduate education in the field of obstetrics and gynecology. Many new procedures, new biomechanical tools aid used in our dailly practice but adoption of these by the residents and specialists in the field should be fast and standardized. This mandates research on medical education, to publish learning curves of emerging procedures for both decreasing possible patient harm and to improve licensing for different procedures. Any research in this area are wellcomed by the editorial board.

There is increasing number of scientific journals in our country. A total of 426 journals are included in Turkish Citation Index. Despite this vast number of journals only 50 of them are indexed by SCI-expanded[1]. Even the journals included in SCI-expanded the highest impact factor by 2017 was 1.6[2]. In order to improve our journals reputation we look forward for any high impact original research.

Thanks in advance.

Sincerely

Eray Çalışkan

Editor in Chief

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Reproductive outcomes of office hysteroscopic metroplasty in women with unexplained infertility with dysmorphic uterus

Açıklanamayan infertilite ve dismorfik uterus olan hastalarda ofis histeroskopik metroplastinin üreme sonuçları

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Abstract

Objective: The correlation between dysmorphic uterus and infertility still remains enigmatic. We evaluated the reproductive outcomes of metroplasty via office hysteroscopy in unexplained infertile women with dysmorphic uteri.

Materials and Methods: In this retrospective cohort study, metroplasty via office hysteroscopy using a bipolar system was performed to 272 women with unexplained infertility with dysmorphic uteri from January 2013 to January 2016. Of all the patients, 162 had primary infertility, and 110 had secondary infertility.

Results: In the primary infertility group, the clinical pregnancy rate was 45.68% (74/162) and the live birth rate was 38.9% (63/162), and in the secondary infertility group, the clinical pregnancy rate was 55.45% (61/110) and the live birth rate was 49% (54/110) after metroplasty. In the secondary infertility group, the miscarriage rate and especially the ectopic pregnancy rate declined dramatically [from 84.5% (93/110) to 9.8% (6/61) and from 15.5% (17/110) to 1.6% (1/61), respectively] ($p<0.01$).

Conclusion: Reproductive outcome can be impaired by Müllerian anomalies, hence, infertile women with dysmorphic uteri should undergo hysteroscopy to improve reproductive outcomes. Our study demonstrated that office hysteroscopic metroplasty of a dysmorphic uterus might improve fertility, particularly in patients with unexplained infertility with dysmorphic uteri, which was an ignored factor previously. Office hysteroscopy is an alternative option in terms of non-invasive procedure.

Keywords: Dysmorphic uterus, T-shaped uterus, office hysteroscopy, obstetric outcome, unexplained infertility

Öz

Giriş: Dismorfik uterus ve infertilite arasındaki ilişki hala bilinmemektedir. Dismorfik uterusu ve açıklanamayan infertilite tanısı olan hastalarda ofis histeroskopik metroplastinin üreme üzerine olan sonuçlarını değerlendirdik.

Gereç ve Yöntemler: Bu retrospektif kohort çalışmada, kliniğimizde Ocak 2013-Aralık 2016 tarihleri arasında bipolar ofis histeroskopik metroplasti uygulanan 272 dismorfik uterus ve açıklanamayan infertil hastalar değerlendirilmiştir. Bu hastaların 162 tanesi primer infertil 110 tanesi sekonder infertildir.

Bulgular: Primer infertil olan hastalarda hsiteroskopik metroplasti sonrası klinik gebelik oranı %45,68 (74/162), sekonder infertil grupta %55,65 (61/110) olarak bulunmuştur. Her iki grupta metroplasti sonrası canlı doğum oranı %38,9 (63/162) ve %49 (54/110)'dur. Düşük oranı ve özellikle dış gebelik oranı sekonder infertil grupta dramatik olarak azalmıştır [düşük oranı; %84,5'den (93/110) %9,8'e (6/61) düşmüş ve ektopik gebelik %15,5'den (17/110) %1,6'ya (1/61) düşmüştür) ($p=0,01$)].

Sonuç: Dismorfik uteruslu infertil kadın fertilitesini artırmak için ofis histeroskopi yoluyla opere edilmelidir. Müllerian anomaliler üreme sonuçlarına olumsuz etkileri olmaktadır. Dismorfik uterusun histeroskopik metroplasti ile düzeltilmesi bizim çalışma grubumuzda özellikle atlanmış uterin faktör olarak infertilite problemini de düzeltebilecektir. Ofis histeroskopi bu işlemin non-invaziv alternatifidir.

Anahtar Kelimeler: Dismorfik uterus, açıklanamayan infertilite, T-şeklinde rahim, ofis histeroskopi, gebelik sonucu

PRECIS: The correlation between dysmorphic uterus and infertility still remains enigmatic. We evaluated the reproductive outcomes of office hysteroscopic metroplasty in unexplained infertile women with dysmorphic uterus. Our study demonstrated that office hysteroscopic metroplasty of a dysmorphic uterus might improve fertility, particularly in unexplained infertility patients with dysmorphic uterus.

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Introduction

A dysmorphic uterus, which was formerly known as "T-shaped uterus" in the American Fertility Society classification of anomalies of the Müllerian duct, is denoted as a second-class (Class U1) uterine anomaly in the European Society of Human Reproduction and Embryology (ESHRE) and the European Society for Gynaecological Endoscopy (ESGE) (ESHRE/ESGE) consensus on the classification of congenital genital tract anomalies, and it leads to poor reproductive and obstetric outcomes[1,2]. Although the prevalence of dysmorphic uterus is not yet known, the reproductive performance of a dysmorphic uterus after hysteroscopic metroplasty is poorly documented; only five trials have been reported to date[3-7]. In the past, operative hysteroscopy for metroplasty was used to expand the uterine cavity; currently, office hysteroscopy with a bipolar system is used, which appears to have minimally invasive effects, thus making it feasible for metroplasty[7]. The encouraging results of the case series of 114 women remain in small numbers in the literature[3-7]. The correlation between dysmorphic uterus and infertility still remains enigmatic. No study has definitely defined such a uterus as being the primary reason for infertility. Although the poor obstetric outcomes of Müllerian anomalies are well-known issues, their reproductive performance in terms of infertility has been relatively ignored. Nevertheless, the lesser expansion of a unicornuate uterus is seen as the cause for infertility. The relationship between a dysmorphic uterus and infertility is not clarified exactly in the literature due to the absence of evidence on this topic. The aim of our study was to evaluate the effectiveness of hysteroscopic metroplasty on reproductive outcome in women with unexplained infertility with dysmorphic uteri.

Materials and Methods

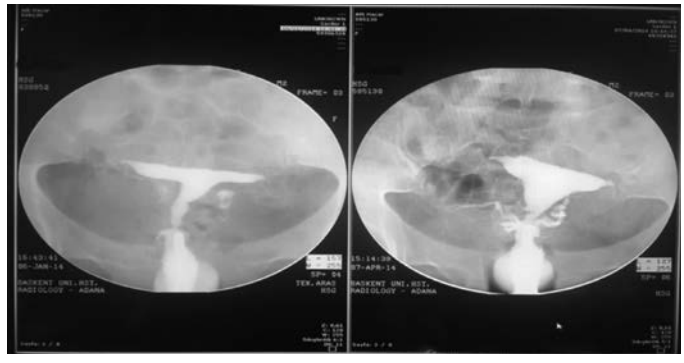
This retrospective study was conducted at Başkent University, Adana Dr. Turgut Noyan Practice and Research Hospital, Clinic of Obstetrics and Gynecology, Division of Reproductive Medicine and In Vitro Fertilization (IVF) Unit. Records of women who underwent hysteroscopic metroplasty for dysmorphic uterus with unexplained infertility from January 2013 to January 2016 were investigated. Mild and/or severe male infertility factor, diminished ovarian reserve, history of endometriosis and/or endometrioma, and tubal pathologies, including bilateral tubal obstruction or hydrosalpinx were excluded and eligible women with unexplained infertility were analyzed. All of the included women were assessed in two groups as primary and secondary infertility. Institutional review board approval This study was approved by Başkent University Institutional Review Board and Ethics Committee approval (KA: 17/151-A) and supported by Başkent University Research Fond was obtained for this retrospective cohort study.

Pre-operative diagnosis

Infertile couples were admitted to Başkent University Division of Reproductive Medicine and IVF Unit. Medical history and ovarian reserve measured using the antral follicle count were evaluated, and hysterosalpingography (HSG) for the uterine cavity and tubal patency and sperm analysis were conducted. Unexplained infertility was defined as follows: unprotected intercourse for at least 1 year without conceiving with a normal ovarian reserve (i.e., antral follicle count over 4 in at least one functioning ovary with a regular menstrual cycle) and bilateral tubal patency observed on HSG and normal sperm analysis based on the World Health Organization 2010 criteria[8]. Dysmorphic uterus, formerly known as "T-shaped uterus," was defined as a condensed lateral side uterine cavity observed using a properly performed HSG, in which the traction to the uterus was sufficient to expose the normal plain of the uterine cavity (Picture 1). The schematic view of the uterus revealed a T-shape, and the angle of the narrowed side wall was almost 90° (Picture 1). We were unable to perform 3D ultrasonography owing to the lack of this technology in our institution. There was no history of diethylstilbestrol (DES) exposure owing to the absence of DES use in Turkey. We think that the relationship between DES exposure and dysmorphic uterus remains speculative. Another issue associated with dysmorphic uterus class U1b, which is the infantile type, was that it could not be evaluated using HSG. This was a limitation of HSG. However, we did not experience any lateral wall perforation.

Operation

Sedation with proper a dosage of propofol and midazolam was applied to the women. After vaginal scrubbing with a povidone-iodine solution, the office hysteroscope was inserted in to the uterine cavity using a non-touch technique. Office hysteroscopy was performed at the early follicular phase (days 6-14) using a 5 mm diameter continuous-flow hysteroscope with an oval profile and a 30° fore-oblique telescope and a 5-Fr operating channel (Office Continuous Flow Operative Hysteroscopy 'size 5'; Karl Storz, Tuttlingen,



Picture 1. Preoperative and postoperative hysterosalpingography image

HSG: Hysterosalpingography

Germany). Cavity distension was induced using an electronic irrigation and aspiration system (Endomat; Karl Storz, Tuttlingen, Germany) with NaCl 0.9%. A stable intrauterine pressure of ~40 mm Hg was obtained by setting the flow rate to 220-350 mm Hg; the negative pressure suction was set at 0.2 bar, and the pressure of irrigation was set at 100 mm Hg. After the evaluation of the uterine cavity, the lateral side walls were cut using a Bipolar Versapoint System (Gynecare Versapoint Bipolar Electrosurgery System, Ethicon, US) until the tubal orifice was visible from the isthmus. The video can be viewed at https://youtu.be/wVh9m0dxB2I. The operations were performed by four experienced surgeons. At the end of the operation, no anti-adhesive gel and/or intrauterine balloon was used. We did not record our operation duration; nevertheless, the mean duration of the operations was ~5 minutes.

Post-operative approach

After the operation, the patients were discharged on the same day. The patients were prescribed 2 mg estradiol valerate oral estrogen support for 3 weeks (Cyclo-progynova tb, Bayer, Turkey). After 2 menstrual cycles, HSG was repeated for the evaluation of the newly formed uterine cavity (Picture 1). All patients were counseled to prevent them from conceiving until the second HSG. After evaluation of the new cavity using the second HSG, all patients were advised to try to conceive without assisted reproductive technology (ART) support for at least 1 year.

Follow-up for the evaluation of reproductive outcomes

The reproductive outcomes were recorded using the delivery registry from our hospital database and via telephone interviews. Clinical pregnancy (CP) was defined as pregnancy diagnosed through ultrasonographic visualization of one or more gestational sacs or a positive blood human chorionic gonadotropin test result. The live birth rate (LBR) was noted as the delivery of at least one live born baby after 24 complete weeks of gestational age. The spontaneous abortion rate was defined as the spontaneous loss of a CP before 20 weeks of completed gestation. The delivery rate ending with live births at full term (37 weeks' gestation) was also recorded.

Statistical Analysis

The data are expressed as mean ± standard deviation. The baseline differences between the groups were analyzed using Student's t-test. Pearson's chi-square test and Fisher's exact test were used to compare the ratios between the groups. Time to pregnancy after metroplasty was measured using Kaplan-Meier survival analysis. A p value of <0.05 was considered statistically significant. The data were analyzed using the SPSS for Windows (version 23.0; SPSS, Inc., Chicago, IL).

Results

During the 3-year study period, 685 office hysteroscopies for dysmorphic uterus were performed to 272 women with unexplained infertility by our IVF department. The baseline characteristics of the patients are shown in Table 1. Of all the patients, 162 had primary infertility, and 110 had secondary infertility. The mean age and mean duration of infertility of the patients were similar. Forty-five women in the primary infertility group and 27 women in the secondary infertility group underwent an IVF/intracytoplasmic sperm injection (ICSI) cycle before metroplasty [27.8% (45/162) and 24.5% (27/110), respectively] (Table 1). In primary infertility group, 45 patients had IVF failures; 23 patients had a history of one cycle failure, 16 had two IVF/CSI failure cycles, and six patients had 3 IVF/ICSI failure cycles before metroplasty. In the secondary infertility group, 27 patients had a history of ART failures; 15 women had one IVF/ICSI failure, eight patients had two failure cycles, and four had three failure cycles. Seven of nine spontaneous pregnancies after metroplasty in the primary infertile women group had just one IVF failure and two patients had two failed IVF/ICSI cycles. In secondary infertile group, the number of spontaneous pregnancy after metroplasty in previous IVF failure cycles was six, four of which had one previous failure and two had 2 IVF/ICSI failures. The CPR and LBR after metroplasty are shown in Table 2. In the secondary infertility group, the miscarriage rate and ectopic pregnancy rate declined dramatically (p=0.01) (Table 2). Nine out of 45 patients (20%) in the primary infertility group and six out of 27 patients (22.2%) in the secondary infertility group with a

Table 1. Basal characteristics of the study groups

	Primary infertility (n=162)	Secondary infertility (n=110)	p
Age	30.53±4.5	30.41±4.66	0.36
Duration of infertility	5.28±3.54	5.71±3.93	0.84
Previous IVF failure number	27.8% (45/162)	24.5% (27/110)	0.57
Spontaneous LBR after metroplasty	18.5% (30/162)	33.6% (37/110)	0.004
IVF/ICSI LBR after metroplasty	20.4% (33/162)	21.8% (24/110)	0.19
Spontaneous LBR after metroplasty with previous IVF failure	20% (9/45)	22.2% (6/27)	0.52

IVF: In vitro fertilization, ICSI: Intracytoplasmic sperm injection, LBR: Live birth rate

Table 2. The reproductive outcomes of the secondary infertility group

	Preoperative	Primary infertility (n=162)	Secondary infertility (n=110)	p
Pregnancy rate		45.7% (74/162)	55.4% (61/110)	-
Miscarriage	84.5% (93/110)	13.5% (10/74)	9.8% (6/61)	<0.001
Ectopic pregnancy	15.5% (17/110)	1.4% (1/74)	1.6% (1/61)	<0.001
Preterm delivery	0	2.7% (2/74)	0	NS
Term delivery	0	82.4% (61/74)	88.5% (54/61)	<0.001
Time to pregnancy (months)	-	6.2±3.2	6.3±4.0	0.94

NS: Not significant

history of IVF/ICSI failures successfully delivered subsequent to metroplasty through spontaneous pregnancies (Table 1). After metroplasty, the pregnancies of 44 patients in the primary infertility group and 24 patients in the secondary infertility group were successful with the first fresh IVF/ICSI cycle without waiting for spontaneous pregnancy. After the second HSG, the time to pregnancy was 5.8±4.7 months in the spontaneous pregnancy group and 6.7±5.7 months in the pregnancies with ART group (Figure1, 2). The time to pregnancy in the primary and secondary infertile groups was also similar (Table 2).

Discussion

Our study demonstrated that office hysteroscopic metroplasty of dysmorphic uterus might improve fertility, particularly in patients with unexplained infertility and with dysmorphic uterus, which was an ignored factor previously. Müllerian anomalies can impair obstetric outcomes because it can cause recurrent pregnancy loss (5-10%), as well as late miscarriage and preterm delivery (25%)[9,10]. These adverse obstetric outcomes may be subsequent to restricted expansion of an abnormally small endometrial cavity[11]. Increased contractility and decreased vascular perfusion of the fibrous uterine septum may also contribute[12]. We hypothesize that the enlargement of the uterine cavity using hysteroscopic metroplasty might restore obstetric outcomes by increasing the possibility for embryo implantation by proper endometrial vascular perfusion together with decreased uterine contractility inducing fragility in a dysmorphic uterus. This hypothesis might be supported by the majority of metroplasty outcomes of uterine septum surgery even in an arcuate uterus in ART cycles[13,14]. The higher prevalence of previous ectopic pregnancy in women with dysmorphic uterus was first reported by Fernandez et al.[6]. Our study also demonstrated that women with secondary infertility with dysmorphic uteri have higher ectopic pregnancy rates (15.5%). After correction of the uterine cavity, the ectopic pregnancy rate dropped to the expected population. This increased rate of ectopic pregnancy might be explained by the increased uterine contractility and decreased endometrial

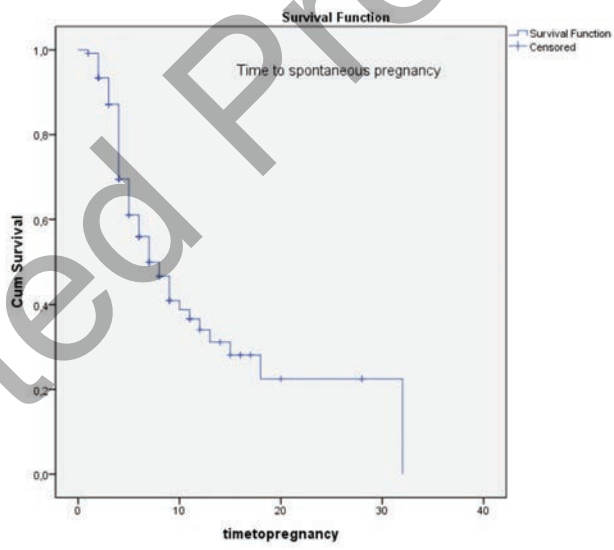


Figure 1. Time to spontaneous pregnancy

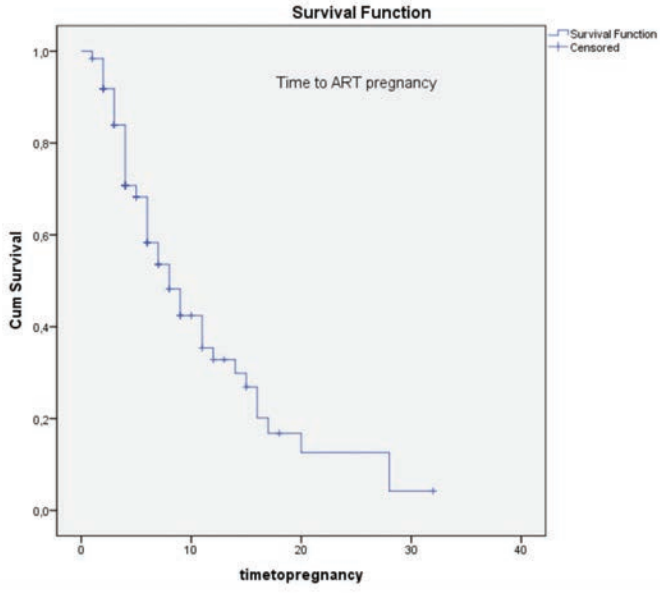


Figure 2. Time to assisted reproductive technology pregnancy

implantation in a dysmorphic uterus. Due to a lack of adequate and credible data, we did not apply any adhesion prevention strategies, such as intrauterine gel or balloon. We evaluated the newly-formed cavity configuration using a second HSG and hence did not observe any adhesions. We did not encounter any complications from hypotonic solution use such as electrolyte changes and hyponatremia because we applied saline during metroplasty procedures. Throughout the 3 years of the study, we did not encounter any complications of hysteroscopy such as uterine perforation or excessive bleeding after the operation. As expected, there would be no further risk of cervical incompetency owing to the thinner hysteroscope, thus ruling out the need for cervical dilatation. The major limitation of this retrospective study is the absence of the confirmation of a narrowed cavity using 3D transvaginal ultrasonography. Although our diagnostic criterion was based only on HSG, despite HSG being superior for the confirmation of dysmorphic uterus, 3D ultrasonography was feasible and appeared to be accurate as. If we had the opportunity to evaluate dysmorphic uterus using 3D ultrasonography, we would have calculated the volume of the endometrial cavity and provided a cut-off volume to compare with that of fertile women's cavities. Although the importance of lateral wall thickness in dysmorphic uterus was included in the ESHRE/ESGE classification, the definitive ratio or thickness in the current literature was not mentioned. Furthermore, there is high intra/inter-observer variability in 3D ultrasonographic measurements of uterine wall thickness for uterine anomalies with conflicting data for their measurements owing to the inadequacy of the technique[15]. Although the ESHRE/ESGE consensus definitive diagnostic criteria were demonstrated using the schematic view for dysmorphic uterus, we speculate that as a diagnostic tool, the combination of HSG and 3D ultrasonography will achieve significantly better outcomes, which might be highlighted by future trials. Our department is an endoscopic surgery and ART clinic to which women who have a potentially higher prevalence of uterine anomalies are referred[16]. This is why the population ratio in this study seemed to be relatively higher. Furthermore, DES exposure was not documented in these women due to the absence of DES marketing in Turkey. In terms of obstetric outcomes, the risk associated with hysteroscopic metroplasty of dysmorphic uterus is the potential for placental adhesion owing to the possibility of electrosurgical injuries to the endometrium and myometrium. We had three different cases of placenta accreta that could be related to the intrauterine operation during the cesarean process. It is possible to see this type of potential adhesion even in the history of cesarean procedures conducted to date, hysteroscopic myomectomy, or any type of endomyometrial injury. The resultant outcomes at our department have been encouraging for fertility expectation in women with dysmorphic uterus. The ignored uterine-factor infertility must be considered in terms of dysmorphic uterus. The relationship between intrauterine adhesion and infertility seems to be similar to dysmorphic uterus related to lesser cavity volumes[17]. Further, the subsiding rate of ectopic pregnancy and miscarriage is also optimizing evidence for enlargement of dysmorphic cavities. Future randomized controlled trials are needed to support the effectiveness of the operation.

Conclusion

Infertile women with dysmorphic uterus should be operated by hysteroscope to improve reproductive outcomes. Office hysteroscope is an alternative option in terms of non-invasive procedure.

Ethics

Ethics Committee Approval: This study was approved by Başkent University Institutional Review Board and Ethics Committee (KA: 17/151A) and supported by Başkent University Research Fond.

Informed Consent: Consent form was filled out by all patients.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.H., E.B.K., T.Ç., P.Ç.A., Concept: B.H., E.B.K., Design: B.H., G.D.D., Data Collection or Processing: G.D.D., S.Ş., Analysis or Interpretation: B.H., Literature Search: B.H., Writing: B.H., G.D.D.

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

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The use of in vitro maturation in stimulated antagonist in vitro fertilization cycles of normo-hyperresponder women due to arrested follicular development: A rescue procedure

Folikül gelişim arresti yaşayan uyarılmış antogonist in vitro fertilizasyon siklusundaki normo-hiperresponder kadınlarda in vitro matürasyonun kullanılması: Bir kurtarma prosedürü

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Abstract

Objective: To evaluate the impact of rescue in vitro maturation (IVM) on the clinical outcomes of women with arrested follicular development in stimulated in vitro fertilization (IVF) cycles

Materials and Methods: This is a retrospective review of 13 patients who were evaluated as normo-hyperresponders for ovarian stimulation. The main outcome measure was the clinical pregnancy and livebirth rates. The purpose of gonadotropin stimulation in patients undergoing IVF is to retrieve multiple oocytes by avoiding multifetal gestation and Ovarian Hyperstimulation syndrome (OHSS). The ovarian response to stimulation ranges from poor response to OHSS, which is related to the follicular number and the dose of the gonadotropins used. However, in some cycles of normo-hyperresponder women, follicular development decelerates or ceases. Close follow-up in a daily manner and increasing the dose of gonadotropins did not change the follicular arrest. This clinical situation has two edges; one is cycle cancellation, which has undesired psychological outcomes for women and the IVF team, and second one is the prolongation of the IVF cycle. For such circumstances, IVM may be a valuable option. Stimulated IVF cycles were converted to IVM as a rescue IVM procedure following detailed informed consent of the women who were close to cycle cancellation.

Results: Thirteen 13 IVM cycles and their clinical outcomes are presented. Six women achieved pregnancies, but only 4 delivered 5 healthy live born. The other two women had biochemical loss during follow-up.

Conclusion: Based on the data obtained, it can be concluded that gonadotropin-stimulated cycles with follicular arrest at the edge of cancellation can be shifted to rescue IVM procedures with reasonable clinical outcomes.

Keywords: Rescue, in vitro maturation, cycle cancellation, in vitro fertilization, pregnancy rate

Öz

Giriş: Uyarılmış in vitro fertilizasyon (IVF) sikluslarında folikül gelişim arresti yaşayan kadınlarda rescue in vitro matürasyonun (IVM) klinik sonuçları üzerine etkisini değerlendirmek

Gereç ve Yöntemler: Bu çalışma over uyarılması için normo-hiperresponder kabul edilen 13 olgunun retrospektif değerlendirilmesidir. Çalışmanın temel çıkarımı klinik gebelik ve canlı doğum oranlarıdır. IVF tedavisindeki hastalarda gonadotropin uyarısının amacı çoğul gebelik ve Yumurtalık Hiperstimülasyon sendromunu (OHSS) önlerken çok sayıda yumurta elde etmektir. Uyarıya yumurtalık yanıtı çok zayıf yanıttan çok aşırı uyarılmaya kadar değişebilmektedir ki bu durum folikül sayısına ve verilen ilacın dozuna bağlıdır. Bununla birlikte normo-hiperresponder bazı kadınlarda folikül gelişimi yavaşlamakta veya durmaktadır. Günlük olarak yakın folikül takibi veya gonadotropin doz artırılması foliküllerdeki arresti değiştirmemektedir. Bu klinik durumun iki ucu vardır; ilki siklus iptalidir ve hem hasta hem de IVF ekibi için istenmeyen psikolojik sonuçları olmaktadır, ikincisi ise IVF siklusunu uzatmaktır. Bu tür durumlar için IVM değerli bir seçenek olabilir. Uyarılmış IVF siklusları, siklus iptalı kıyısında olan hastalardan detalı yazılı onam alındıktan sonra kurtarma

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IVM prosedürüne dönüştürülmüştür.
Bulgular: Burada 13 IVM siklusu ve klinik sonuçları sunulmuştur. On üç kadından 6'sında gebelik elde edilmiş, 2 kadın biyokimyasal kayıp yaşarken, 4 kadın 5 sağlıklı bebek doğurmuştur.
Sonuç: Elde edilen veriler temelinde, iptalin eşiğinde olan foliküler arrest gelişmiş uyarılmış siklusları kurtarma IVM prosedürüne dönüştürlebilir ve kabul edilebilir gebelik sonuçları vardır.
Anahtar Kelimeler: Kurtarma, in vitro matürasyon, siklus iptali, in vitro fertilizasyon, gebelik oranı

PRECIS: Rescue in vitro maturation for arrested follicular development in stimulated cycles.

Introduction

The story of in vitro fertilization (IVF) began with immature oocytes from unstimulated cycles and finally succeeded with the birth of Louise Brown in 1978[1]; however, the roots of studies about immature oocytes and in vitro oocyte maturation (IVM) go back to the 1930s[2]. Clomiphene citrate (CC) was discovered in the 1960s and following the livebirths after IVF, the first stimulations were made with CC and multifollicular development in IVF cycles were achieved. Later the addition of human menopausal gonadotropins eased the course of IVF and increased the success rates, but also increased drug-related life-threatening complications such as Ovarian Hyperstimulation syndrome (OHSS). Tremendous use of gonadotropins resulted in the advent of recombinant drugs used in IVF practice. In late the 1990s and early 2000s, the discussion was around reverting to natural or semi-natural cycle managements[3-5]. In the 1990s, IVM babies were born and IVM gained attention in assisted reproductive technologies (ART)[6,7]. To date, the total number of babies born from IVM is around 5000, which cannot be compared to the huge number of babies born from conventional IVF (>7 million). This means that IVM carries some controversies and is not a first-choice treatment in ART[8]. The most susceptible women for IVM treatment are patients with Polycystic Ovarian syndrome (PCOS); the best clinical outcomes in IVM cycles were obtained in patients with PCOS[8]. The second most common reason for using IVM is to avoid OHSS because patients with PCOS are vulnerable to OHSS[9]. IVM has been studied extensively in women PCOS, but indications other than PCOS such as normoresponder women, poor responder women, fertility preservation, rescue IVM for preventing OHSS in stimulated cycles[10], oocyte maturation problems, and patients with cancer who need urgent fertility preservation were introduced into IVM practice[11-15]. This study is the first to present IVM shifted from the conventional stimulated IVF cycles due to arrested follicular growth in order to rescue cycles from cancellation. Thirteen normo-hyperresponder patients whose gonadotropin antagonist stimulation cycles were shifted to IVM with human chorionic gonadotropin (hCG) priming were evaluated. All follicles arrested 12 mm and less in size and antagonist drugs were not used in any of the patients. Among the 13 patients, 6 pregnancies were achieved, 2 pregnancies were lost as biochemical pregnancies and the remaining 4 patients delivered five babies with good health. The clinical outcomes of this rescue IVM is acceptable and promising.

Materials and Methods

This is a retrospective case series of 13 women who underwent rescue IVM therapy at Clinart IVF Center, a private center in the Trabzon province of Turkey, between May 2011 and September 2014. Detailed informed consents of the patients were signed and registered. Institutional Review Board Clinart International Hospital approval by a grant number of 000280/18.05.2015 is present for this trial. For the normo and hyper-responder patients included in the study, a gonadotropin-antagonist protocol was the preferred treatment for ovarian stimulation. The drugs used were: Gonal-F flacon 150-300 IU subcutaneous (sc) once daily (recombinant follitropin alfa, Merck Serono, Switzerland) as recombinant follicle stimulating hormone (FSH) and Cetrotide 0.25 mg sc once daily (cetrorelix acetate 0.25 mg for sc injection, Merck Serono, Switzerland) as gonadotropin-releasing hormone (GnRH) antagonist. Women whose husbands had severe oligoasthenoteratospermia, azoospermia, or cryptozoospermia were excluded. Each patient underwent ovarian stimulation therapy for 5 to 6 days, in regards to mentioned protocol. However, GnRH antagonists could not be administered because of arrested follicular response. Patients with inadequate follicular growth or follicular arrest were given detailed information about the rescue IVM treatment as a valuable option, instead of cycle cancellation. In patients who approved rescue IVM therapy, oocyte retrieval was performed 36 hours after hCG priming with 10.000-20.000 IU/IM. The length of the cycles was similar to FSH priming IVM but shorter than stimulated cycles. Oocyte retrievals of the patients were performed via a 16-gauge double-lumen aspiration needle (Swemed by Vitrolife, Sweden) with low-pressure continuous flushing and collected in a heparinized collection medium. There are no established criteria to identify the ideal timing or method for oocyte retrieval, with most studies using a lead-follicular diameter of up to 12 mm. Lead-follicle diameters greater than 13 mm have been associated with reduced numbers of collected and matured oocytes, possibly related to subsequent atresia of the non-dominant follicles from withdrawal of endogenous FSH support. The aspiration technique for immature oocytes

also differs compared with conventional IVF. Transvaginal ultrasound-guided oocyte collection was performed with an aspiration pressure of 100 mmHg. Although the pressure is usually set between 50 and 80 mmHg, in this protocol the pressure was increased to a maximum 100 mmHg instead of prolonging the time of pick-up with low pressure. Extremely high aspiration pressure has been shown to have a negative impact on oocyte development. It takes approximately 10 minutes for oocyte pick-up, and 10 minutes for the evaluation of oocytes per patient. No complications were reported in the oocyte pick-up procedure and all patients were discharged on the same day. The spermatozoa for intracytoplasmic sperm injection (ICSI) were prepared using a three-layer PureSperm gradient (Codes PSB-100 and PS-100-100, Mölndal, Gothenburg, Sweden). The whole medium used for ICSI was prepared and incubated for one day prior to the procedure. Ten milliliters of flushing medium without heparin, and 10 mL of paraffin in Falcon flasks were incubated at 37 °C in atmosphere with high humidity without gas, and a Falcon Center-well dish containing a total of 4 mL of universal IVF medium (Medi-Cult, Code 10311010A) including 1 mL in the center and 3 mL in the perimeter covered with liquid paraffin was incubated at 37 °C in an atmosphere of 6% CO_2 and 5% O_2 with high humidity. In addition, a Falcon Petri dish with 40-50 µL droplets of interstellar medium (ISM) 1 medium (Medi-Cult, Code 10500010A) covered with 7 mL liquid paraffin was incubated at 37 °C in an atmosphere of 6% CO_2 and 5% O_2 with high humidity. For denudation of the oocytes, 0.7 mL flushing medium without heparin was placed in each well of the four-well dish and covered with liquid paraffin. Eleven microliters of HYASE 100 medium (Vitrolife, Code 10017, 5x0.1 mL, Kungsbacka, Sweden) was added in 1 well of the dish. Removal of the cumulus and corona cells was performed in hyaluronidase-containing medium using Pasteur pipettes after a 26-to-28 hour incubation period. There is no consensus as to which formulation is best suited for the purpose of *in vitro* oocyte culture. The oocytes were then transferred to universal-IVF medium for culture. All ICSI procedures were performed in a Falcon Petri dish with droplets of polyvinylpyrrolidone-containing medium for sperm (Vitrolife, Code 10111, 5x0.1 mL) and droplets of flushing medium without heparin for oocytes. After the ICSI procedure, the oocytes were placed into a ISM 1 medium for culture. In most cases, the fertilized embryos were transferred into the uterine cavity on days 2 or 3. The luteal phase was supported with vaginal progesterone (Progestan 200 mg tablet, Koçak Pharmacy, İstanbul, Turkey) administration once daily and 100 µg transdermal estradiol (Estraderm TTS, Novartis Pharma AG, Basel, Sweden) administration once daily until the fetal heart beat was detected.

Statistical Analysis

Since this is a case series study without comparisons, we used only excel for analyzing the datas.

Results

The demographic characteristics, clinical and laboratory parameters are listed in Table 1. The mean age of the women included in the study was 28.3 years with a maximum of 33 and minimum of 24 years. The mean infertility time was 5.4 years. The mean antral follicle count on the third day of the menstrual cycle was 10 in the whole group. Anti-müllerian hormone (AMH) levels were measured in 5 mL venous blood drawn specifically for the present study using enzyme-linked immunosorbent assay. The mean AMH level was 4.79 ng/dL with a maximum of 6.4 and minimum of 2.6 ng/dL. The mean endometrial thickness was 9.9 mm. The mean retrieved oocyte count was 11.15, and the mean retrieval time was 14.4 minutes. The mean number of obtained metaphase II (MII) oocyte was 7.6. Although only one embryo was transferred to 10 of the 13 patients, two embryos were transferred to the remaining 3 patients. Pregnancy was achieved in six patients, two of these six pregnancies were biochemical pregnancies. The remaining four pregnancies resulted in birth of five healthy babies.

Discussion

The present study shows that IVM converted from stimulated antagonist IVF cycles may be a good alternative approach with favorable outcomes in normo-hyperresponder women whose follicles are resistant to stimulation by gonadotropins. Cycle cancellation due to frustrated follicular growth in normo-hyperresponder patients is an undesirable condition for couples and physicians, and it is not a commonly observed clinical situation. Before cancelling the cycle, day-to-day monitoring or dose oscillations can be used to overcome the follicular arrest. However, in some cases, follicles still resist from growing. Rescue IVM can be offered in such cases to save the cycles from cancellation after giving thorough information about the procedure to couples. It is obvious that, all of the oocytes retrieved in an IVF cycle are not mature, regardless of the chosen IVF protocol. In almost all retrievals, germinal vesicle or MI oocytes were observed, which are discarded from ICSI procedures. IVM has lost its value over time because the clinical outcomes, number of matured oocytes, and developmental competence of embryos are not as good as those obtained from stimulated cycles. Recent advances in ovarian stimulation procedures, safer protocols aimed at decreasing the risk of OHSS, have decreased the attention on IVM[16,17]. However, accumulating data from articles supporting IVM because it is safe, cost-effective, simple, repeatable, flexible, and patient friendly in nature, without the risk of OHSS, it serves as a good treatment option. As such, IVF specialists need to be encouraged to add IVM to their clinical practice instead of neglecting it[18-21]. There are few publications regarding rescue IVM in the literature. Jaroudi et al.[22] were the first to report 3 cases in which IVM was used to secure cycles in poor responders, which resulted

Table 1. Clinical characteristics of 13 patients

Case number	Age	Duration of infertility (year)	D3 AFC	D3 AMH	Endometrial thickness on the day of HCG (mm)	Number of oocytes retrieved	Oocyte retrieval duration (min.)	Number of MII oocytes	Number of transferred embryos	Grade of transferred embryos	Total dose and duration of Gonadotropin (IU/day)	Outcome
1	25	5	12	4.7	11	7	14	5	1	G2	1500/6	BHCG (-)
2	33	3	10	6.05	10	12	14	10	1	G2	1500/6	BHCG (-)
3	24	3	8	4.1	7.8	10	15	7	1	G2	1500/6	BHCG (-)
4	29	2.5	7	3.6	9.7	8	15	7	1	G2	1350/6	BHCG (-)
5	28	5	8	4.7	9.2	11	15	7	2	G2-G3	1350/6	Live birth 1
6	31	2	7	4.1	9	9	15	7	1	G2	1500/6	Live birth 1
7	33	8	9	2.6	8.4	11	15	4	2	G2-G2	1350/6	Live birth twin
8	24	1	12	6.4	13	11	13	7	1	G2	1500/6	BHCG (-)
9	30	2	12	6.2	10.4	13	14	9	1	G2	1350/6	BHCG (-)
10	28	12	12	4.2	13.5	9	14	7	1	G2	1500/6	BHCG (-)
11	30	9	9	4.4	9.7	8	12	8	1	G2	1500/6	Biochemical pregnancy
12	24	13	13	6.3	7.6	20	17	12	1	G2	1500/6	Biochemical pregnancy
13	29	5	12	5	10	16	15	10	2	G2-G2	1500/6	Live birth 1

AFC: Antral follicle count, AMH: Anti-müllerian hormone, HCG: Human chorionic gonadotropin, BHCG: Beta human chorionic gonadotropin, MII: Metaphase II, Min.: Minimum

in deliveries of healthy babies. In a case report from Turkey, Yalcınkaya et al.[23] studied IVM in a poor responder patient who achieved pregnancy and concluded that IVM could be used in poor responders as a good alternative. Braga et al.[24] from Brazil used IVM to mature immature oocytes derived from stimulated cycles but they found that rescue spontaneous maturation of the oocytes did not contribute to clinical pregnancy rates in poor responder women. IVM was studied in patients with repeated oocyte maturation problems from Empty Follicle syndrome (EFS) to oocyte maturation arrest. Patients with Genuine-EFS syndrome (G-EFS) benefited from IVM cycles and achieved ongoing pregnancies[25]. In a case report of a woman with a history of repeated G-EFS and azoospermia in her husband, oocytes were retrieved and injected with Mic-TESE derived sperms (DC1); a healthy embryo was transferred but pregnancy was not achieved[26]. Another interesting case report revealed that in Resistant Ovary syndrome, IVM worked and oocyte retrieval, embryonic development, and a successful delivery was achieved[27]. For managing OHSS, IVM converted from antagonist stimulation cycles were preferred and early hCG priming when the leading follicles were less than 14 mm was planned and favorable laboratory and clinical outcomes were achieved[11]. That study resembles our study, but their problem was hyper-response, whereas in our study, the main problem was poor ovarian response in normo-hyperresponsive women. IVM was compared with IVF-ICSI procedures concerning miscarriage rates and Buckett et al.[28] reported that pregnancy loss and clinical miscarriage rates after IVM was higher compared with IVF-ICSI, but this situation was related to PCOS rather than IVM. The oocyte is the central part of folliculogenesis and follicular growth cannot be separated from oocyte development. The oocyte's journey to maturation is an extraordinary processes and until recently, granulosa cells were thought to be the main contributor of oocyte growth, but the oocyte itself seems to be the key factor in maturation. Two oocyte-derived factors, growth differentiation factor-9 and bone morphogenetic protein-15, moderate regulatory functions and play an important role in oocyte-granulosa cells interaction[29-31]. This means that any problem in the follicular environment may interfere with oocyte maturation and also follicular maturation. Apoptotic factors found in the follicular fluids of women with G-EFS explain the early oocyte loss in the follicles, thus IVM remains the treatment of choice in patients with G-EFS. FSH and hCG priming alone in IVM cycles are less successful than FSH and hCG priming together in these cycles. Although the results for FSH and hCG priming together are conflicting in IVM, this approach increases the maturation and fertilization rates and developmental competence when compared with other IVM modalities[32]. However, Child et al.[33] studied IVM in unstimulated cycles, cycles primed with FSH, and cycles primed with both FSH and hCG in PCOS, and reported similar maturation, fertilization, and cleavage potential in all IVM modalities. IVM treatment shifted from stimulated antagonist IVF cycles seems like FSH and hCG priming IVM. The only difference is the selected dose at the beginning of the cycle. Similarly, favorable pregnancy results with insemination of IVM oocytes from unstimulated cycles were obtained in a study conducted by Söderström-Anttila et al.[34]. In hCG-priming-alone IVM cycles, *in vitro*-matured oocytes have more multinucleation and worse clinical outcomes when compared with FSH-priming-alone cycles[35]. However, other studies revealed that priming with FSH alone or FSH with hCG priming together had good embryologic and clinical outcomes compared with unstimulated IVM cycles[36]. In another article, Fadini et al.[37] showed that FSH priming together with hCG priming in IVM cycles had better clinical outcomes compared with FSH priming or hCG priming alone.

Conclusion

hCG-priming IVM can be a good option for women experiencing follicular resistance to gonadotropins in antagonist cycles. By this modality, cycles can be rescued from cancellation with favorable clinical outcomes. Nevertheless, information on the safety of IVM with regard to malformation and developmental outcomes cannot be assessed accurately because only a small number of children have been conceived with IVM.

Ethics

Ethics Committee Approval: This study is approved by the local ethical committee of Clinart International Hospital (approval number: 000298/18.05.2015).

Informed Consent: Written informed consent were taken from each participating patients.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.S.H., Ş.H., Concept: Ş.H., E.H., M.H.D., Design: Ş.H., E.S.H., S.A., A.Z.I., Data Collection or Processing: Ş.H., S.A., M.H.D., Analysis or Interpretation: M.H.D., Ş.H., E.S.H., A.Z.I., Literature Search: Ş.H., E.S.H., S.A., Writing: Ş.H., S.A., M.H.D.

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A retrospective comparative study of prednisolone use in antagonist co-treated assisted reproductive technology cycles for patients with good prognosis

Yardımcı üreme teknikleri ile tedavi edilecek iyi prognozlu hastaların antagonist sikluslarında prednizolon kullanımı retrospektif karşılaştırma

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Abstract

Objective: To investigate the impact of peri-implantation prednisolone use and its duration in antagonist co-treated assisted reproductive technology (ART) cycles of patients with good prognosis.

Materials and Methods: Infertile patients treated with gonadotropin-releasing hormone antagonist protocol between January 2010 and June 2013 were included. The patients in group A (n=196) received no prednisolone. The patients in groups B (n=397) and C (n=371) received 5 mg oral prednisolone daily, for 4 and 12 days following embryo transfer, respectively. The main outcome parameter was live birth rate.

Results: The ages of the groups were 30.1±4.6, 31.5±4.5, and 30.9±4.7 years, respectively (p=0.163). There was no statistically significant difference between the groups regarding cycle characteristics. Implantation rates were 20.7%, 24.6%, and 23.8%, respectively (p=0.163). Miscarriage rates were 1.5%, 3.5%, and 3.2%, respectively (p=0.859). Live birth rates were 28.7%, 29.3%, and 32.8%, respectively (p=0.482).

Conclusion: Empiric prednisolone administration during the peri-implantation period does not seem to have beneficial effects in ART cycles of patients with good prognosis.

Keywords: Assisted reproductive technology, glucocorticoid, gonadotropin-releasing hormone antagonist, peri-implantation period

Öz

Amaç: Yardımcı üreme teknikleri (YÜT) ile tedavi edilecek iyi prognozlu hastaların antagonist sikluslarında peri-implantasyon döneminde prednizolon kullanımının etkisinin araştırılması.

Gereç ve Yöntemler: Ocak 2010 ile Haziran 2013 arasında gonadotropin releasing hormon antagonist protoklü ile tedavi edilen infertil hastalar dahil edilmiştir. A grubundaki hastalar (n=196) prednizolon kullanmamıştır. B grubundaki (n=397) ve C grubundaki (n=371) hastalar embriyo transferini takiben sırasıyla 4 gün ve 12 gün 5 mg oral prednizolon kullanmıştır. Ana sonuç parametresi canlı doğum oranıdır.

Bulgular: Grupların yaş ortalamaları sırasıyla 30,1±4,6, 31,5±4,5 ve 30,9±4,7 yıldı (p=0,163). Siklus özellikleri açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu. Düşük oranları sırasıyla %1,5, %3,5, ve %3,2 olarak bulundu (p=0,859). Canlı doğum oranları sırasıyla %28,7, %29,3 ve %32,8 olarak bulundu (p=0,482).

Sonuç: İyi prognozlu hastaların YÜT sikluslarında peri-implantasyon döneminde ampirik prednizolon kullanımının olumlu etkisi yoktur.

Anahtar Kelimeler: Yardımcı üreme teknikleri, glukokortikoid, gonadotropin releasing hormon antagonisti, peri-implantasyon dönemi

PRECIS: In the present study, we evaluated peri-implantation prednisolone use and its duration in a large cohort of patients undergoing assisted reproductive technology with good prognosis.

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Introduction

The implantation process of the embryo is a consequence of complex molecular interactions involving many cytokines, growth factors, and immune cells[1,2]. In this regard, several molecules have been suggested to improve implantation and contribute to successful pregnancy when administered during the peri-implantation period. Glucocorticoids, well-known agents with anti-inflammatory and immune suppressive properties, have been investigated for the last few decades and conflicting data have been published[3-8]. Some authors advocate the beneficial effect in zona-dissected embryos and in the presence of assisted-hatching, whereas others reported significantly higher pregnancies in women with auto-antibodies after the use of glucocorticoids[6,9-11]. On the contrary, several researchers reported no significant beneficial effect of peri-implantation glucocorticoid administration on implantation and clinical pregnancy rates in intracytoplasmic sperm injection (ICSI) cycles[12,13]. Published evidence is too limited and heterogeneous to support any firm conclusion on the value of preimplantation prednisolone use in assisted reproductive technology (ART) for patients considered to have good prognosis. In the present study, we aimed to investigate the impact of peri-implantation prednisolone use and its duration in antagonist co-treated ART cycles of patients with good prognosis.

Materials and Methods

Infertile patients treated with gonadotropin-releasing hormone (GnRH) antagonist co-treated ART in the Infertility Centre of Ankara University Faculty of Medicine, Turkey, between January 2010 and June 2013 were included in this retrospective cohort study. The clinic where the present study was conducted belongs to a tertiary referral hospital that mainly serves the central and east side of the country with approximately 1000 ART cycles per year. The Institutional Review Board of Ankara University Faculty of Medicine approved the study (approval number: 08-341-16). The first stimulation cycle for each subject was included in the study to prevent possible crossover bias between the groups. The inclusion criteria were being female, age 18-40 years, baseline follicle-stimulating hormone (FSH) level <15 IU/L, diagnosed as tubal factor or unexplained infertility, ICSI treatment, and with complete birth data. The exclusion criteria were body mass index (BMI) >30 kg/m^2, presence of any untreated thyroid dysfunction/hyperprolactinemia, diminished ovarian reserve according to the Bologna criteria[14] or premature ovarian failure, uterine abnormality, positive tests for antinuclear, anti-double-stranded DNA, anticardiolipin antibodies or lupus anticoagulant, male factor infertility, endometriosis, frozen-thaw cycles, cycles managed with assisted hatching, and cycles with day 5 embryo transfer (ET). Cycle cancellations were performed due to a lack of ovarian response or fertilization failure. For eligible participants, we extracted all data regarding controlled ovarian stimulation (COS) and clinical outcomes from the database, and divided the patients into three groups according to their prednisolone administration protocol. Group A received no prednisolone. Groups B and C received 5 mg oral prednisolone daily for 4 and 12 days following ET, respectively. The different prednisolone protocols were due to the primary physician's choice. Ovarian stimulation was performed with recombinant FSH (Gonal-F, Merck-Serono, İstanbul, Turkey) beginning from the second day of the menstrual cycle with a fixed starting dosage of 150 IU/day. Dose adjustment was performed individually according to ovarian response. The GnRH antagonist (Cetrotide, Merck-Serono, İstanbul, Turkey) was introduced (0.25 mg/day) on the sixth day (fixed antagonist protocol) and continued throughout ovarian stimulation. When at least three follicles were ≥18 mm, recombinant human chorionic gonadotropin (hCG) 250 µg (Ovitrelle, Merck-Serono, İstanbul, Turkey) was used for final oocyte maturation. Transvaginal ultrasonography-guided oocyte pick-up (OPU) was performed 35-36 hours after the hCG trigger. ET was performed on the 3rd day of OPU. A maximum of two embryos were transferred under ultrasound guidance due to national ET regulations[15]. Embryos on the 2nd and 3rd days were classified as cleavage stage embryos and were graded based on cell numbers and the degree of fragmentation. All women were administered luteal phase support through 90 mg/day vaginal micronized progesterone (Crinone 8% gel; Merck-Serono, İstanbul, Turkey) commenced on OPU day. In the event of pregnancy, luteal phase support was continued until 10 weeks of gestation. Pregnancy and clinical pregnancy were defined, respectively, by measuring serum β-hCG levels 2 weeks after ET and as the presence of heartbeat at 6-7 weeks of gestation. The implantation rate was calculated separately for each woman as the number of gestational sacs divided by the number of transferred embryos multiplied by 100. The primary outcome measure was live birth rate (LBR).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, United States) 15.0 for Windows software was used for all statistical analyses. The Shapiro-Wilk test was used to test normal distribution of continuous parameters. When distribution of a continuous variable was normal, parametric tests were preferred. Continuous variables were compared using the One-Way ANOVA test. Categorical variables were compared using the chi-square test. Continuous data where descriptive tests used are presented as mean ± standard deviation and categorical data are presented as frequency (percentage). A p value of <0.05 was considered statistically significant. While planning the present study, we were not able to detect any previous studies investigating the effect of peri-implantation period use of prednisolone and its dosage

in ART cycles of patients with good prognosis. Hypothetically, when a power analysis was performed with 80% power and an α value of 0.05 for an approximately 5% difference in LBR per cycle, the patient number for each study arm should be 1328 for the confirmation of statistical significance. Thus, in the present study, type 2 statistical error could not be excluded for this parameter. Considering the difficulty of recruiting so many participants to a single-centre trial, the aim was to finish the current trial using the data of the available cohort such that it could be included in future meta-analyses on the issue.

Results

During the study period, a total of 2970 ART cycles were performed in our unit. Among those, 1226 were first ART cycles of tubal factor or unexplained infertility patients, among whom 78 (6.3%) patients with BMI >30 kg/m^2 and 184 (15%) patients with frozen-thawed or day 5 ET were excluded. As a result, the data of 964 first ART cycles of patients with good prognosis were found eligible for assessment.

The demographic characteristics of the study and control groups are presented in Table 1 and the cycle characteristics of the groups are presented in Table 2. The outcome measures of the study are presented in Table 3. There were no statistically significant differences between the groups regarding clinical pregnancy, ongoing pregnancy, and LBRs.

Discussion

The aim of the present study was to investigate the impact of peri-implantation prednisolone use and its duration in antagonist co-treated ART cycles of patients with good prognosis. We found no significant impact of prednisolone administration during the peri-implantation period and its duration on implantation and clinical pregnancy rates and LBR in antagonist co-treated ART cycles of patients with good prognosis. To the best of our knowledge, this analysis is the largest evaluation of the effect of peri-implantation prednisolone use in antagonist co-treated cycles, and is the only comparison of two different doses of prednisolone. Immune suppressive properties of glucocorticoids have been questioned in terms of enhancing outcomes when administered during peri-implantation period because several factors are effective on implantation process. Although several studies and meta-analyses reported beneficial effects on pregnancy rates, those studies included patients with recurrent miscarriages[16-18]. However, in our

Table 1. Demographic characteristics of the study groups

	Group A (n=196)	Group B (n=397)	Group C (n=371)	p
Age (years)[a]	30.1±4.6	31.5±4.5	30.9±4.7	0.065
Duration of infertility (years)[a]	6.2±1.6	6.8±3.9	7.0±3.8	0.253
Basal Estradiol (pg/mL)[a]	46.7±35.9	51.9±33.7	48.5±26.3	0.020[b]
Basal FSH (mIU/mL)[a]	7.5±2.6	7.8±3.5	7.9±4.7	0.605

Group A received no prednisolone, group B received prednisolone for 4 days, group C received prednisolone for 12 days. [a]: All values are expressed as mean ± standard deviation, statistical analysis was performed using the One-Way ANOVA test, [b]: The significance stems from the difference between groups A and B (p<0.05), FSH: Follicle-stimulating hormone

Table 2. Cycle characteristics of the study groups

	Group A (n=196)	Group B (n=397)	Group C (n=371)	p
Duration of stimulation (days)[a]	10.4±3.6	10.4±2.3	10.8±2.1	0.051
Total dose of gonadotropins (IU)[a]	2338±868	2471±827	2350±787	0.067
Max E_2 (pg/mL)[a]	2306±1179	2001±1204	1829±1137	<0.001[b]
Retrieved oocytes (n)[a]	9.0±5.7	8.8±6.1	8.4±5.2	0.492
MII oocytes (n)[a]	6.8±4.5	6.8±5.2	6.8±4.6	0.996
Fertilization rate (%)[a]	67.4±29.3	62.6±28.7	63.7±32.6	0.051
Endometrial thickness on day of OPU (mm)[a]	10.5±1.7	10.2±1.5	10.1±2.4	0.058
No of transferred embryos (n)[a]	1.5±0.7	1.5±0.8	1.5±0.8	0.522
Implantation rate (%)[a]	20.7±35	24.6±35.6	23.8±33	0.163

Group A received no prednisolone, group B received prednisolone for 4 days, group C received prednisolone for 12 days. [a]:All values are expressed as mean ± standard deviation. Statistical analysis was performed using the One-Way ANOVA test, [b]: The significance stems from the differences between groups A and B (p<0.01), Groups A and C (p<0.01) and groups B and C (p<0.01), E_2: Estradiol, OPU: Oocyte pick-up, MII: Metaphase

Table 3. Comparison of outcome measures

	Group A (n=196)	Group B (n=397)	Group C (n=371)	p
Clinical pregnancy, n (%)[a]	59 (33.1%)	135 (34.0%)	144 (39.2%)	0.263
Ongoing pregnancy, n (%)[a]	56 (31.5%)	121 (30.6%)	132 (35.7%)	0.292
Live birth, n (%)[a]	51 (28.7%)	116 (29.3%)	120 (32.8%)	0.482
Miscarriage, n (%)[a]	3 (1.5%)	14 (3.5%)	12 (3.2%)	0.859
Cycle cancellation, n (%)[a]	16 (8.2%)	25 (6.4%)	20 (5.6%)	0.487

study, we investigated the effect of prednisolone on patients with good prognosis. In the meta-analysis by Boomsma et al.[7] including 14 RCTs and 1879 couples, the empiric use of prednisolone during the peri-implantation period was assessed and a borderline statistically significant increase in pregnancy rates was reported in in vitro fertilization but not in ICSI cycles, suggesting its limited use in ICSI cycles. Despite these results, the authors made their conclusions with caution because the included trials in which ICSI was used were very few and clinically heterogeneous. However, in our study, we included only first ICSI cycles of patients with good prognosis to obtain a relatively homogenous cohort. In the present study, we investigated prednisolone 5 mg because lower doses have already been reported to reveal similar immune-suppression and pregnancy outcomes when compared with higher doses[12,19,20]. According to the results of our study, short and long-term use of prednisolone has a similar effect on implantation and pregnancy rates. Additionally, only antagonist co-treated cycles were included because this protocol has widely replaced GnRH agonist cycles globally with its applicability and non-inferior outcomes. The implantation and pregnancy rates were consistent with rates in the available literature, especially with those of Ubaldi et al.[12] who also included patients with good prognosis and used low-dose glucocorticoid. The large number of subjects included in the analyses and the strict inclusion criteria of those with good prognosis were the main strengths of our study. The available LBR data might be of some interest. Moreover, we assessed the impact of prednisolone duration, comparing short and prolonged use. According to the results, neither short nor long-course peri-implantation-period prednisolone administration has any benefit in antagonist co-treated ART cycles of patients with good prognosis. Hence, prednisolone should not be prescribed for routine ART cycles. The results of our study may be used in future meta-analyses investigating prednisolone administration in ART cycles of patients with good prognosis. Large randomized clinical trials may be more suggestive on prednisolone use in patients undergoing ICSI with good prognosis.

The retrospective nature and lack of randomization are the main limitations of the present study. Another limitation is the different manipulations during the COS protocols, mainly dose adjustment and duration of prednisolone treatment, due to primary physician preferences, which could affect the outcome. Moreover, the low power of the statistical analysis can be noted as a limitation. However, given the select nature of our population, attaining such a large cohort was unrealistic in a single-centre study.

Conclusion

In the present study we could not find an unequivocal beneficial effect of empiric prednisolone administration during the peri-implantation period in women undergoing their first ART cycle with an antagonist protocol. According to the results of our study, within the context of its limitations, a complete shift in clinical practice cannot be suggested.

Ethics

Ethics Committee Approval: The study was approved by the Ankara University Local Ethics Committee (approval number: 08-341-16).

Informed Consent: Not applicable.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ö., M.S., B.B., C.S.A., Concept: B.Ö., E.G.P., Design: B.Ö., Y.E.Ş., Data Collection or Processing: H.U., E.G.P., Analysis or Interpretation: C.S.A., B.Ö., Y.E.Ş., Literature Search: Y.E.Ş., H.U., E.G.P., Writing: Y.E.Ş., H.U., E.G.P., B.Ö.

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Circulating SCUBE1 levels in women with polycystic ovary syndrome

Polikistik over sendromlu kadınlarda SCUBE1 düzeyi

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is thought to represent an early manifestation of metabolic syndrome, which is associated with cardiovascular disease. Signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-epidermal growth factor domain-containing protein 1 (SCUBE1) is a platelet activation marker that plays important roles in vascular biology and has been closely linked to cardiovascular events. In the present study, we investigated SCUBE1 levels in lean glucose-tolerant women with PCOS and assessed the possible association between SCUBE1 levels and hormonal and metabolic features of women with PCOS.

Materials and Methods: The study population consisted of 90 lean [body mass index (BMI) <25 kg/m^2] women who were diagnosed as having PCOS using the Rotterdam criteria and 100 age- and BMI-matched healthy controls with no clinical or biochemical feature of hyperandrogenism. Glucose tolerance was evaluated in all subjects before recruitment using the 2 h 75 g oral glucose tolerance test, and only those exhibiting normal glucose tolerance were enrolled. Hormonal and metabolic parameters, and serum SCUBE1 levels were evaluated.

Results: Circulating SCUBE1 levels were significantly higher in women with PCOS than in controls (5.9±3.9 vs. 4.2±1.4 ng/mL, p=0.022). No association between SCUBE1 level and clinical or biochemical parameters was found in the control or PCOS group.

Conclusion: SCUBE1 levels are elevated in women with PCOS compared with those in healthy controls; thus, this protein may be an early biomarker of cardiovascular disease later in life.

Keywords: Platelet activation, Polycystic ovary syndrome, SCUBE1

Öz

Amaç: Polikistik over sendromu (PKOS), kardiyovasküler hastalıkla ilişkili olan metabolik sendromun erken bulgusu olarak düşünülmektedir. Signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-epidermal büyüme faktörü domain-containing protein 1 (SCUBE1) vasküler biyolojide önemli rol oynayan ve kardiyovasküler olaylarla yakından ilişkili olan bir trombosit aktivasyon belirtecidir. Bu çalışmada, normal kilo ve glukoz toleransı olan PKOS'li kadınlarda SCUBE1 düzeyleri ve bu düzeylerin hormonal ve metabolik özellikler arasındaki olası ilişkinin değerlendirmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya normal kiloya sahip [vücut kitle indeksi (VKİ) <25 kg/m^2], Rotterdam kriterlerine göre PKOS tanısı konulan 90 hasta ile yaş ve VKİ eşleştirilmiş klinik ve biyokimyasal hiperandrojenizm bulgusu olmayan 100 kontrol hastası dahil edildi. Tüm olgulara 2 saatlik 75 gr oral glukoz tolerans testi uygulandı ve sadece normal glukoz toleransı olan olgular çalışmaya dahil edildi. Hormonal ve metabolik parametreler, serum SCUBE1 düzeyleri değerlendirildi.

Bulgular: SCUBE1 seviyeleri, PKOS'li kadınlarda kontrol grubuna göre anlamlı olarak daha yüksek saptandı (5,9±3,9'a karşı 4,2±1,4 ng/mL, p=0,022). Kontrol veya PKOS grubunda SCUBE1 seviyesi ile klinik veya biyokimyasal parametreler arasında ilişki bulunmadı.

Sonuç: PKOS'li kadınlarda SCUBE1 seviyeleri, sağlıklı kontrol grubundakilere göre daha yüksektir; bu nedenle, bu protein yaşamın ileri döneminde gelişebilecek kardiyovasküler hastalıkların erken biyobelirteci olabilir.

Anahtar Kelimeler: Trombosit aktivasyonu, Polikistik over sendromu, SCUBE1

PRECIS: Circulating SCUBE1 levels are elevated in women with Polycystic ovary syndrome compared with those in healthy controls; thus, this protein may be an early biomarker of cardiovascular disease later in life.

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, and is a heterogeneous clinical condition characterized by hyperandrogenism and signs of chronic oligo-/anovulation. The prevalence of PCOS ranges from 6% to 10%, depending on the criteria used, ethnicity, and geographic location[1]. Although originally considered to be a gynaecologic disorder, PCOS is associated with reproductive and metabolic disturbances, including ovulatory dysfunction, hyperandrogenism, dyslipidaemia, increased insulin resistance, and impaired glucose intolerance. Furthermore, PCOS is thought to represent an early manifestation of metabolic syndrome, which is associated with cardiovascular disease[2]. Several cardiovascular risk factors, such as dyslipidaemia, impaired fibrinolysis, chronic low-grade inflammation, endothelial dysfunction, and subclinical and clinical atherosclerosis, are more prevalent in women with PCOS[3]. In addition, these risks factors are strongly linked to insulin resistance and are compounded by the common occurrence of obesity. Data regarding the long-term cardiovascular consequences of PCOS are conflicting[4].

Signal peptide-CUB (complement C1r/C1s, Uegf, bone morphogenetic protein-1)-epidermal growth factor-like protein (SCUBE) is an evolutionarily conserved gene family composed of three different isoforms (SCUBE1-3)[5]. SCUBE family members are secretory membrane proteins that play important roles during mouse embryogenesis by regulating extracellular signal transport, molecular adhesion, and migration[6,7]. Although SCUBE1 was originally isolated from vascular endothelial cells, it is stored predominantly in alpha granules of inactive platelets in humans[8,9]. Following platelet activation, SCUBE1 expression is up-regulated and SCUBE1 is translocated to the cell surface, cleaved, and released into circulation in small soluble particles. These circulating fragments enhance platelet-platelet adhesion and agglutination under thrombotic conditions. SCUBE1 accumulates in platelet-rich thrombus and atherosclerotic vascular lesions[9]. An experimental study showed that genetic loss or functional neutralisation of soluble SCUBE1 prevents thrombosis[10]. In addition, a single nucleotide polymorphism of the *SCUBE1* gene is associated with enhanced risk of venous thromboembolism[11]. Taken together, these data suggest that SCUBE1 is involved in modulating vascular biology. In this study, we aimed to investigate SCUBE1 levels in lean glucose-tolerant women with PCOS, and to assess the possible associations between SCUBE1 levels and the hormonal and metabolic features of this syndrome.

Materials and Methods

This prospective case-control study was conducted at the Antalya Training and Research Hospital, Antalya, Turkey, between June 2015 and March 2016. The Ethics Committee of the institution approved this study, and all subjects provided written informed consent. A total of 190 lean [body mass index [BMI] <25 kg/m^2] patients, aged 20-35 years, with normal glucose tolerance (NGT), were recruited from the outpatient gynaecology clinic of our institution. The study group consisted of 90 women diagnosed with PCOS using the revised 2003 Rotterdam consensus criteria as the presence of two of the following three features: 1) oligomenorrhea (inter-menstrual interval >35 days) or amenorrhea (absence of menstruation for 3 consecutive months), 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries as revealed by typical imaging features on ultrasonographic examination (12 or more follicles 2-9 mm in diameter in each ovary and/or ovarian volume >10 cm^3)[12]. One hundred healthy women with no clinical or biochemical features of hyperandrogenism were recruited as the control group. All of the control subjects were ovulatory as evidenced by regular menstruation (lasting 21 to 35 days) and luteal-phase serum progesterone levels >5 ng/mL. The PCOS and control groups were matched in terms of both age and BMI. Glucose tolerance was evaluated before study recruitment using the criteria of the American Diabetes Association[13]. Thus, the 2 h 75 g oral glucose tolerance test (OGTT) was administered to all subjects, and only those with NGT were enrolled. NGT was defined as a fasting glucose level <100 mg/dL or a 2 h glucose level <140 mg/dL. Exclusion criteria were impaired glucose regulation, diabetes mellitus (DM), hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, acromegaly, hypothalamic disorder, hypertension, systemic inflammatory disease, any vascular disorder, coagulation abnormalities, history of alcohol consumption or smoking, and family history of DM and/or PCOS. No participant had taken any medication known to affect hormone, lipid, or carbohydrate metabolism (e.g. insulin-sensitising drugs, oral contraceptives, anti-androgens, corticosteroids, statins, or aspirin) within the previous 3 months. Late-onset congenital adrenal hyperplasia was excluded by measuring a normal 17-hydroxyprogesterone level (<1.2 ng/mL during the early follicular phase) in a baseline morning blood sample.

Anthropomorphic and clinical measurements

As part of the physical examination, weight and height of each patient were recorded, and BMI was calculated using the formula: weight (kg)/height (m^2). Waist and hip circumferences were measured in a standing position with the feet fairly close together. Waist circumference was measured midway between the lower rib margin and the iliac crest, and hip circumference was measured over the maximum circumference of the buttocks, to calculate the waist/hip ratio (WHR). Systolic and diastolic blood pressure of each patient was measured after a 10 min rest and recorded. The modified Ferriman-Gallwey figure was self-scored after all participants were given an explanation and demonstration in full

detail[14]. Hyperandrogenism was defined as clinical hirsutism (modified Ferriman-Gallwey score ≥8), acne, alopecia, and/or an elevated androgen level (total testosterone >0.75 ng/mL (manufacturer's reference range: 0.1-0.75 ng/mL) and/or dehydroepiandrosterone sulphate (DHEAS) >430 µg/dL (manufacturer's reference range: 35-430 µg/dL). Ovarian morphology was evaluated by transvaginal ultrasonography or transabdominal ultrasonography (DC-7, Mindray Medical International Ltd., Shenzhen, China) with a distended bladder in virginal women on the same day that blood samples were obtained.

Biochemical Analysis

Laboratory tests were performed during the early follicular phase (days 3-7 of the menstrual cycle) after a spontaneous bleeding episode, or independent of the cycle phase if amenorrhea was evident. Baseline blood samples were obtained from large forearm antecubital veins after a 12 h overnight fast, and all subjects underwent the standard 2 h 75 g OGTT. The blood samples were placed in plain tubes, stored at room temperature for at least 30 min to allow clotting, and centrifuged at 2500 rpm for 15 min at 4 °C to separate the serum. Concentrations of serum glucose, insulin, and other hormone and lipid parameters were assayed immediately. Additional serum was isolated from fasting blood samples and stored at -80 °C for later analysis of SCUBE1 level. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, and sex hormone-binding globulin (SHBG) levels were determined using a two-site immunoenzymatic method, and DHEAS levels were measured using a competitive binding immunoenzymatic method employing a commercially available kit (Beckman Coulter Diagnostics, Fullerton, CA, USA) and an autoanalyer (Access DxI800; Beckman Coulter). Serum 17-hydroxyprogesterone levels were determined using a commercially available kit (DiaMetra, Segrate, Italy) and an autoanalyzer (Etimax 3000; DiaSorin, Stillwater, MN, USA). Glucose levels were measured using the hexokinase technique and a commercially available kit (Beckham AU5800; Beckham Coulter). Insulin levels were determined using a chemiluminescent assay (AccessDxI800; Beckman Coulter). Serum triglyceride, total cholesterol, high-density lipoprotein, and low-density lipoprotein cholesterol levels were determined using an autoanalyzer (Beckman AU5800; Beckman Coulter). The intra- and inter-assay coefficients of variation (CVs) for all assays were 5% and 10%, respectively.

The free androgen index (FAI) was calculated as total serum testosterone level (nmol/L) ×100/SHBG (nmol/L). We estimated insulin resistance using the homeostatic model assessment-insulin resistance (HOMA-IR) index, defined as fasting plasma insulin value (µU/mL) × fasting plasma glucose value (mg/dL)/405[15]. Insulin sensitivity was calculated using the quantitative insulin-sensitivity check index (QUICKI), according to the following formula: 1/[log (fasting insulin level (µU/mL) + log (fasting glucose level (mg/dL)]][16].

Serum SCUBE1 levels were measured using a commercially available enzyme-linked immunosorbent assay (cat no. E-EL-H5405; Elabscience Biotechnology, Wuhan, China), according to the manufacturer's instructions. Assay sensitivity was 0.38 ng/mL, and the inter- and intra-assay CVs were <10% and 8%, respectively.

Statistical Analysis

The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables are presented as mean ± standard deviation if normally distributed or as median (range) if not normally distributed. Between-group differences were detected using Student's t-test for parametric data and the Mann-Whitney U test for nonparametric data. Correlations between SCUBE1 levels and other parameters were calculated using Pearson's correlation analysis (normally distributed data) or Spearman's rank test (data not normally distributed). Two-sided p values <0.05 were considered to be significant. The statistical analysis was performed using the SPSS ver. 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics and biochemical data of the control subjects and patients with PCOS are presented in Table 1. These parameters were similar between the two groups because the participants were matched in terms of age and BMI (p>0.05). As expected, the hirsutism score was significantly higher in patients with PCOS than in the control group (p<0.001). Obstetric history and WHR did not differ between the groups (p>0.05). Serum levels of LH and total testosterone, as well as the FAI were significantly higher, but serum SHBG level was significantly lower in women with PCOS than in the control group (p<0.05 for all). On the other hand, no differences in FSH, 17-hydroxyprogesterone, DHEAS, insulin, fasting or 2 h post-load glucose concentration, HOMA-IR or QUICKI values or lipid parameters were detected between the two groups. Serum SCUBE1 levels were significantly higher in patients with PCOS than in the controls (5.9±3.9 vs. 4.2±1.4 ng/mL, p=0.022). Serum SCUBE1 levels in patients with PCOS stratified according to hyperandrogenism were not statistically different from one another (5.8±2.8 ng/mL in normoandrogenic PCOS vs. 5.9±3.1 ng/mL in hyperandrogenic PCOS, p=0.91). No significant correlation was found between SCUBE1 concentrations and any clinical or biochemical parameters in either group (Table 2).

Discussion

Our results show that serum levels of SCUBE1, a platelet activation marker, were significantly higher in young, lean glucose-tolerant women with PCOS than in age- and BMI-matched healthy controls. Moreover, no significant correlations were detected between any hormonal or metabolic

Table 1. Clinical and laboratory features of the control and Polycystic ovary syndrome groups

	Control (n=100)	PCOS (n=90)	p value
Age (years)	26.6±5.1	25.1±4.5	0.118
Anthropometric measurements			
BMI (kg/m^2)	22.1±1.9	22.3±1.7	0 .467
WHR	0.74±0.04	0.75±0.03	0.737
Systolic blood pressure (mm Hg)	117.3±5.8	120.4±9.5	0.45
Diastolic blood pressure (mm Hg)	70.8±5.3	74.6±3.5	0.52
Hirsutism score	5.1±1.9	14.2±7.1	<0.001*
Hormonal components			
FSH (mIU/mL)	5.5±1.7	5.6±1.5	0.774
LH (mIU/mL)	4.5±3.1	10.1±5.4	<0.001*
17-OHP (ng/mL)	0.8±0.3	0.7±0.3	0.127
Androgens			
Total testosterone (ng/mL)	0.4±0.1	0.6±0.2	<0.001*
DHEAS (µg/dL)	230.8±110.8	264.2±112.5	0.117
SHBG (nmol/L)	55.5±26.8	42.1±29.5	<0.001*
Free androgen index	3.1±2.2	7.1±5.7	0.002*
Insulin sensitivity and glucose tolerance			
Fasting insulin (mIU/mL)	8.1±3.7	9.8±5.7	0.247
Fasting glucose (mg/dL)	85.8±6.5	86.1±7.1	0.468
2 h glucose (mg/dL)	88.8±17.4	91.2±17.7	0.392
HOMA-IR	1.7±0.8	2.1±1.2	0.279
QUICKI	0.35±0.03	0.36±0.02	0.301
Lipid profiles			
Triglyceride (mg/dL)	89.8±39.9	98.3±51.9	0.122
Total cholesterol (mg/dL)	160.8±31.8	170.2±32.8	0.133
HDL cholesterol (mg/dL)	62.4±24.4	51.7±12.6	0.111
LDL cholesterol (mg/dL)	89.2±21.5	92.9±20.1	0.064
SCUBE1 (ng/mL)	4.2±1.4	5.9±3.9	0.022*

Values are given as mean ± SD or median (range) as indicated PCOS: Polycystic ovary syndrome, BMI: Body mass index, WHR: Waist-hip ratio, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, 17-OHP: 17-hydroxyprogesterone, DHEAS: Dehydroepiandrosterone sulfate, SHBG: Sex hormone-binding globulin, HOMA-IR: Homeostasis model assessment insulin resistance, QUICKI: Quantitative insulin-sensitivity check index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein
*Significant difference

PCOS parameters and SCUBE1 concentrations. These results suggest that PCOS, in the absence of obesity and glucose intolerance, results in increased platelet activation, even in young women. To the best of our knowledge, this is the first study to evaluate SCUBE1 levels in women with PCOS. Although platelets are involved in fundamental processes of vascular biology, excess platelet activation may lead to platelet-mediated thrombosis and associated clinical ischemic events. Several studies have investigated SCUBE1 as a marker of platelet activation in such patients. Dai et al[17]. reported that SCUBE1 was elevated in patients with acute coronary syndrome and acute ischaemic stroke compared with patients with chronic coronary disease and healthy controls. These findings were corroborated by Sonmez et al.,[18] who reported that the analysis of circulating SCUBE1 levels provided useful diagnostic information to distinguish patients with acute coronary syndrome from those with non-coronary chest pain. Given the relationship between platelet hyperactivity

Table 2. Correlations of SCUBE1 levels with clinical and biochemical parameters in the control and Polycystic ovary syndrome groups

	Control		PCOS	
	r	p value	r	p value
Age	0.036	0.824	0.029	0.856
BMI	0.106	0.458	0.149	0.189
WHR	0.176	0.272	0.049	0.666
Hirsutism score	0.022	0.893	0.163	0.152
FSH	0.078	0.627	0.013	0.909
LH	0.163	0.309	0.018	0.875
17-OHP	0.277	0.079	0.048	0.676
DHEAS	0.014	0.932	0.099	0.387
SHBG	-0.238	0.134	0.164	0.261
Total testosterone	-0.1	0.534	-0.012	0.916
Free androgen index	0.054	0.737	0.129	0.259
Fasting insulin	0.086	0.595	0.034	0.767
Fasting glucose	0.163	0.308	0.136	0.231
2-h glucose	0.035	0.828	0.079	0.491
HOMA-IR	0.075	0.643	0.029	0.798
QUICKI	-0.128	0.424	-0.001	0.995
Total cholesterol	-0.201	0.209	-0.128	0.263
Triglyceride	0.084	0.604	0.199	0.079
HDL cholesterol	-0.089	0.578	-0.054	0.636
LDL cholesterol	-0.248	0.119	-0.001	0.991

PCOS: Polycystic ovary syndrome, BMI: Body mass index, WHR: Waist-hip ratio, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, 17-OHP: 17-hydroxyprogesterone, DHEAS: Dehydroepiandrosterone sulfate, SHBG: Sex hormone-binding globulin, HOMA-IR: Homeostasis model assessment insulin resistance, QUICKI: Quantitative insulin-sensitivity check index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

and cardiovascular events, extensive interest has developed regarding platelet function in women with PCOS. Dereli et al.[19] demonstrated higher platelet aggregation induced by adenosine diphosphate (ADP), collagen, and epinephrine in a cohort of lean women with PCOS compared with those in age- and BMI-matched controls. Of interest, platelet aggregation was negatively correlated with insulin sensitivity. Rajendran et al.[20] found more ADP-induced platelet aggregation and less platelet responsiveness to the inhibitory effects of nitric oxide in lean and obese women with PCOS than in age-matched controls. These authors suggested that hyperandrogenism was responsible for impaired platelet function in women with PCOS because no difference in platelet aggregation was demonstrated between the lean and obese PCOS groups. In contrast, Kahal et al.[21] found no difference in baseline platelet function, ADP-induced platelet aggregation, or platelet responsiveness to the inhibitory effects of prostacyclin between obese women with PCOS and BMI-matched controls. These equivocal data can be explained by the use of different PCOS diagnostic criteria and techniques to assess platelet function. In addition to the *in vitro* studies mentioned above, several research groups have investigated *in vivo* platelet activation biomarkers, such as P-selectin and platelet-derived microparticles (PMPs), in patients with PCOS. P-selectin is an adhesion molecule secreted extracellularly from platelet alpha granules that is involved in platelet aggregation. Yildiz et al.[22] demonstrated that soluble P-selectin levels were significantly higher in young, normal glucose-tolerant women with PCOS than in age-and BMI-matched healthy controls. On the other hand, no significant correlation was observed between soluble P-selectin levels and any anthropometric or biochemical parameter in the PCOS group. PMPs are small vesicles released from the surface of activated or apoptotic platelets as a result of membrane remodelling. PMPs are highly procoagulant due to expression of phospholipids and tissue factors on their outer membranes, which are the main

initiators of the coagulation cascade. Koiou et al.[23,24] reported higher circulating PMP levels in lean and overweight/obese women with PCOS compared with those in BMI-matched controls. PMPs were correlated with serum testosterone levels [23] and the mean number of ovarian follicles[24].

In the present study, serum total testosterone levels and FAI were significantly higher in women with PCOS than in controls. Therefore, we speculate that higher SCUBE1 levels in women with PCOS may be attributable to hyperandrogenaemia. However, we failed to show any correlation between SCUBE1 and total testosterone level, FAI, or any other clinical or biochemical parameter in either the control or PCOS groups. The reason for these results is linked to the fact that we included only lean and normal glucose-tolerant subjects to avoid any confounding effects of obesity and/or impaired glucose tolerance on platelet function[25,26]. Low-grade systemic inflammatory activation in patients with PCOS may contribute, at least in part, to increased SCUBE1 level[27]. Indeed, SCUBE1 levels increase in response to stimulation by proinflammatory cytokines, such as interleukin-1β and tumour necrosis factor-α[8]. Taken together, these findings suggest that increased SCUBE1 level is an independent contributor to an increased risk of cardiovascular events in women with PCOS, regardless of other traditional risk factors, such as obesity, insulin resistance, and hyperandrogenism.

Study Limitations

The present study has several limitations. Our study design was cross-sectional in nature, and long-term consequences of increased SCUBE1 level in women with PCOS were not evaluated. Another limitation is that although SCUBE1 is derived mainly from platelets; we did not investigate expression of this marker from those cells. Finally, our study subjects were mostly young women; therefore, our results may not be generalizable to older patients with PCOS.

Conclusion

Circulating SCUBE1 levels are elevated in young, lean, glucose-tolerant women with PCOS compared with those in healthy controls; thus, this protein may be an early biomarker of cardiovascular disease later in life. Additional studies are required to clarify the potential impact of SCUBE1 in the pathogenesis of PCOS and to investigate its association on the cardiovascular risk of these patients.

Ethics

Ethics Comittee Approval: The study was approved by the Local Ethics Committee of Antalya Training and Research Hospital (approval number: 2015-81266704).

Informed Consent: All subjects provided written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contribution

Concept: O.E., Design: O.E., H.Y.E., Data Collection or Processing: M.K.Ö., H.Y.E., Analysis or Interpretation: O.E., A.U.D., Literature Search: E.E., N.Y., Writing: O.E., H.Y.E.

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

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Modified abdominal packing method in "near miss" patients with postpartum hemorrhages

Postpartum kanamalı "near miss" hastalarda modifiye abdominal packing yöntemi

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Abstract

Objective: To describe a more effective abdominal packing method in patients with disseminated intravascular coagulation following peripartum hysterectomy due to postpartum hemorrhage (PPH).

Materials and Methods: The present retrospective and descriptive study was conducted to document six cases with refractory pelvic bleeding who underwent a second surgery for PPH between January 2016 and December 2017 at İstanbul Zeynep Kamil Woman and Children Diseases Training and Research Hospital.

Results: Karateke packing was performed to control intra-abdominal massive hemorrhages of five women who were referred to our clinic due to PPH who had undergone peripartum hysterectomy and hypogastric artery ligation but hemostasis could not be provided. In addition, a case of hypovolemic shock due to placenta percreta rupture in a woman who had also undergone an emergency hysterectomy and hypogastric artery ligation, which had failed. Hemostasis was provided in all patients. No method-related complication developed.

Conclusion: Karateke packing is a very easy method to perform, it is more effective than the classic abdominal packing technique, with a low complication rate, and most importantly, life-saving in patients undergoing a peripartum hysterectomy due to PPH and thereafter experiencing diffuse hemorrhage.

Keywords: Abdominal packing, peripartum hysterectomy, near miss

Öz

Amaç: Postpartum kanama nedeni ile peripartum histerektomi yapılan ancak kanama kontrolü sağlanamayan hastalarda daha etkili bir abdominal packing yöntemi tariflemek.

Gereç ve Yöntemler: İstanbul Zeynep Kamil Kadın ve Çocuk Hastalıkları Eğitim ve Araştırma Hastanesi'nde Ocak 2016-Eylül 2017 tarihleri arasında doğum sonu kanama nedeniyle tekrarlayan cerrahi girişim uygulanan 6 pelvik kanamalı olguyu retrospektif, gözlemsel ve tanımlayıcı olarak inceledik.

Bulgular: Postpartum kanama nedeniyle kliniğimize refere edilen ve peripartum histerektomi ve hipogastrik arter ligasyonu yapılan; ancak kanama kontrolü sağlanamayan beş olgu ile plasenta perkreata rüptürü nedeniyle hipovolemik şokta olan ve acil histerektomi ve hipogastrik arter ligasyonu yapılan bir olgunun intraabdominal masif kanamalarını kontrol etmek için Karateke usulü packing yapıldı. Tüm hastalarda kanama kontrolü sağlandı. Yönteme bağlı komplikasyon gelişmedi.

Sonuç: Sonuç olarak Postpartum kanama nedeniyle peripartum histerektomi yapılan ve sonrasında hayatı tehdit eden diffüz kanamalarda Karateke packing yöntemi uygulanması oldukça kolay, klasik abdominal packing'ten daha etkili, komplikasyonu az ve herşeyden önemlisi hayat kurtaran bir yöntemdir.

Anahtar Kelimeler: Abdominal packing, peripartum histerektomi, near miss

PRECIS: Karateke packing method can be used as the last life-saving method in patients with postpartum hemorrhage.

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Introduction

Postpartum hemorrhage (PPH) is the most common cause of maternal death with high morbidity and mortality rates. The maternal mortality rate has fallen in most countries with advances in reaching blood and blood products, multidisciplinary approaches to cases, and the development of comprehensive protocols. In our country, this rate has fallen from 0.28% to 0.14% in last 10 years[1,2]. To maintain sufficient circulation and tissue oxygen supply in PPH, and to stop the hemorrhage simultaneously, interventions such as uterotonic agents, use of Bakri balloon tamponade, B-lynch suture, uterine artery or hypogastric artery ligation, uterine artery embolization, and when necessary, hysterectomy in addition to general obstetric procedures are life-saving[3,4]. The incidence of peripartum hysterectomy is 0.2/1000 deliveries in developed countries; however, this rate is 4.43/1000 deliveries in developing countries[1]. Despite such efficient treatment options in PPH, repeat laparotomies may be required to control ongoing hemorrhage following a peripartum hysterectomy in patients administered massive transfusion and developing disseminated intravascular coagulopathy (DIC)[5]. Abdominal packing is a classic technique that is frequently performed in these "near miss" patients who have no other surgical option. Abdominal packing exerts a mechanical pressure over the low pressure venous and capillary vessels of intra-abdominal deperitonized pelvic surfaces and the surgery region and saves time for transfusion of necessary coagulation factors and blood and blood products such as platelets required for the provision of permanent hemostasis. Additionally, abdominal packing gains time for the clearance of mediators preventing intravascular coagulation by the kidney and liver, and treatment of metabolic problems such as DIC, hypothermia, acidosis, and hypovolemic shock occurring in a patient in the intensive care unit[6]. As it was indicated in the review of Touhami et al.,[7] the decision for pelvic packing is generally taken in the event of the development of nonsurgically controllable hemorrhage associated with clinical and laboratory evidence of coagulopathy and not being able to replace blood loss sufficiently despite continuous hemorrhage after an emergency peripartum hysterectomy. Accordingly, the procedure of pelvic packing may be considered as a "rescue ending procedure".

Indications for pelvic packing after emergency peripartum hysterectomy

- Hemodynamically unstable patient without optimal resuscitation[8,9]
- Stabilization of the patient to enable transfer to an adequate structure[10]
- Post hysterectomy hemorrhage with a free interval[8,11,12]
- Diffuse vaginal laceration related to pelvic bleeding[13,14]
- Presence of extensive hematoma in the absence of the possibility of embolization[11,14]

In this study, we aimed to describe a new packing method that we considered as a more effective method in hemorrhage cases requiring a peripartum hysterectomy due to PPH after delivery and in "near miss" patients in whom hemostasis could not be provided despite peripartum hysterectomy and who have no other surgical option and require more than one surgery to control the hemorrhage.

Materials and Methods

The present retrospective, observational, and descriptive study was conducted to document patients who had a PPH between January 2016 and December 2017 at Zeynep Kamil Research and Training Hospital. The study protocol was approved by the Zeynep Kamil Women and Children Research Hospital local ethics committee (approval number: 120-2015). Informed consent was provided by all participants. A need for intervention had occurred due to a severe PPH in a total of 310 patients. One hundred sixty-four patients (53%) were referred from external centers. Surgical intervention was performed in the external center in 33 patients. The causes of PPH were as follows: postpartum atony in 212 patients, placental invasion anomalies in 81 patients, a uterine rupture in 7 patients, severe retroperitoneal hematoma secondary to lower genital tract injury in 8 patients, and uterine inversion in 2 patients. The causes of PPH and demographic characteristics of patients are shown in Tables 1 and 2. The following interventions were performed: Bakri balloon tamponade in 50 patients, pelvic devascularization and compression suture in 105 patients, local uterine resection in 33 patients, and peripartum hysterectomy in 122 patients.

Statistical Analysis

A new abdominal packing technique was performed as the final method in six patients undergoing surgery for at least a second time for PPH but not controlled with classic hemostatic methods for whom all surgical methods were exhausted. The data obtained from the study were analyzed using the Statistical Package for the Social Sciences software package (SPSS version 10.0) and the results are expressed as mean ± standard deviation.

Results

The packing technique was performed in five women in whom hemostasis could not be achieved despite a peripartum hysterectomy, and in a patient who presented to the emergency department with the clinical picture of hypovolemic shock due to placenta percreta rupture. Relaparotomy had a severe risk for these 6 "near miss" patients. Therefore, to create a more effective compression considering that sufficient compression could not be provided, a new packing method (Karateke packing) was performed. The packing decision in these patients was made by the obstetric emergency bleeding team of our clinic. The demographic characteristics of these 6

patients are presented in Table 3. The packing technique used by the obstetric bleeding team was performed in the following steps: In Karateke packing, a 1 cm incision is performed in the posterior vaginal wall 1-2 cm from the sutured vaginal cuff, and a Bakri balloon is placed into the abdomen by pulling through the vagina. Six to seven soaked and squeezed near hot sponges are wrapped circularly around the balloon (Figure 1) and then the Bakri balloon is inflated with 500-1000 mL saline and put in traction through the vaginal route (Figure 2). Thus, abdomen of the patient becomes filled with sponges around the inflated Bakri balloon. The balloon exerts compression on the underlying sponges by means of vaginal traction and therefore the pressure is transferred to all surfaces of the surgical region at the same rate and efficiently. In this way, bleeding stops in vascular structures and hemostasis is provided (Figure 3). The distal part of the balloon, under sufficient traction, is fixed to the leg of the patient. Thereafter, the skin is closed without closing the abdominal fascial layers. To prevent Compartment syndrome and perfusion failure of the lower extremities, maintenance perfusion is allowed by releasing the tension on the shaft of the Bakri balloon minimally with 2 hour intervals during the postoperative period. In the event of continuance of bleeding, the balloon is inflated more, more traction is applied, and compression on the underlying sponges is increased. This procedure is continued until hemostasis is provided.

The depacking procedure was performed in all patients after coagulation parameters returned to the normal range

Table 1. Characteristics of the patients who underwent peripartum hysterectomy because of postpartum hemorrhage (n=310)

	Mean
Age	32.2±4.57
Gravida	3
Parity	2
Previous cesarean sections	
No	83 (27%)
Yes	227 (73%)
History of repeat C/S	85 (27%)
Gestational week	36
Mode of delivery	
Cesarean delivery	183 (59%)
Vaginal delivery	127 (41%)
Hospital stay (days)	10±5
Preoperative Hct, %	35 (12-47)
Preoperative platelet count	168742±66800

Hct: Hematocrit, C/S: Cesarean sections

Table 2. The causes of postpartum hemorrhages and patients requiring transfusion

Postpartum hemorrhage	n
Atony	212
Placenta accreta	81
Retroperitoneal hematoma	8
Uterine rupture	7
Uterine inversion	2
Massive blood cell transfusion	69 (5-30 Unit)
Platelet transfusion	32 (8-100 Unit)
Fibrinogen	86 (2-20 g)
rFVIIa*	2 (8-16 g)

*rFVIIa: Recombinant Factor VII a

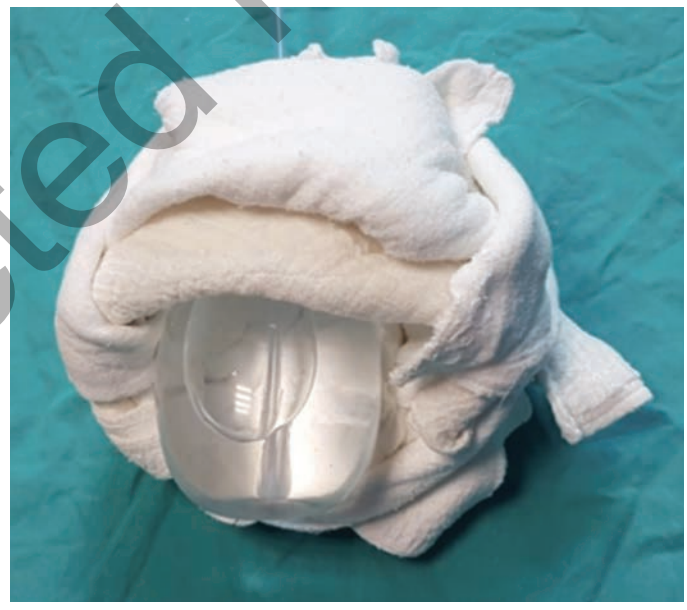

Figure 1. Soaked and squeezed near-hot sponges are wrapped circularly around the balloon

Table 3. Characteristics of the patients requiring multiple surgeries and abdominal packing following peripartum hysterectomy (n=6)

	Mean
Age	33.33±4.27
Gravida	4
Parity	3
Gestational week	37±2
Mode of delivery	
Cesarean delivery	3
Vaginal delivery	3
Hospital stay (days)	10±5

and cessation of intra-abdominal hemorrhage following blood and blood products replacement. Preoperative and postoperative laboratory parameters of all our patients are shown in Table 2. In our first patient who received Karateke packing, bilateral hypogastric artery ligation, and B-Lynch suture were performed via laparotomy due to postpartum uterine atony and there was no intra-operative bleeding. A peripartum hysterectomy was performed with a second laparotomy because 2000 mL/h bleeding occurred through the drain of the patient in the intensive care unit postoperatively. Upon continuance of intra-abdominal diffuse bleeding, classic packing was performed perioperatively with 6 pads and the abdomen was closed. At the postoperative 1st hour, the total drained fluid volume was 1500 cc in the drain bag, and a third laparotomy was performed. Karateke packing was performed as the final intervention because there was no surgical option to control the bleeding in the 3rd surgery. In this patient, blood loss of 500 mL/24 h was observed through the drain in the intensive care unit postoperatively.

Peripartum hysterectomy and hypogastric artery ligation were performed in three of our patients due to postpartum atony and peripartum hysterectomy, and bilateral hypogastric artery ligation was performed in one of our patients due to uterine atony and broad ligament hematoma. Upon observation of severe bleeding through the drains of patients followed up in the intensive care unit postoperatively, Karateke packing was performed in a second surgery.

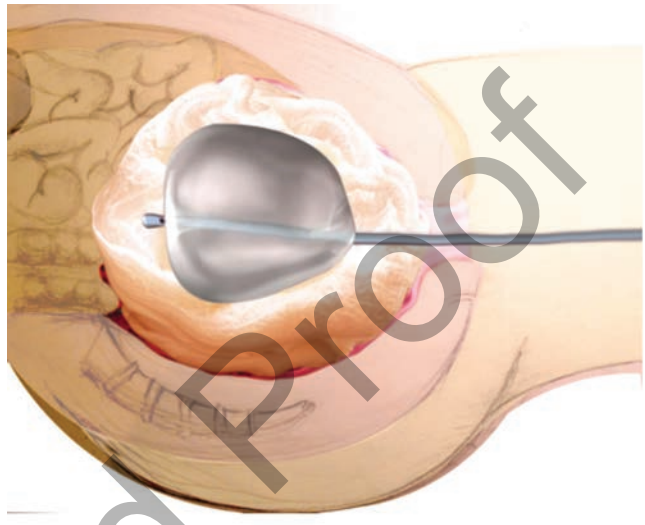

Figure 2. Bakri balloon is inflated with 500-1000 mL saline and put in traction through the vaginal route

Table 4. Clinical features of the patients requiring multiple surgeries and abdominal packing following peripartum hysterectomy (n=6)

Diagnosis	Operations	Bleeding after hysterectomy	Transfused blood products	Complications	Hospital stay (days)
Preeclampsia, abruptio placenta -postpartum atony	1. B-lynch + hypogastric artery ligation 2. Hysterectomy + classic abdominal packing	1300 mL/h	RBC: 16 FFP: 28 Platelet: 100 WBC: 14 Fib: 20	Acute tubular necrosis + Sheehan's syndrome	20
Abruptio placenta-postpartum atony	Hysterectomy + hypogastric artery ligation	1000 mL/h	RBC: 5 FFP: 8 Platelet: 8 Fib: 4	-	6
Postpartum atony	Hysterectomy + hypogastric artery ligation	1500 mL/h	RBC: 12 FFP: 16 Platelet: 32 Fib: 16	Reversible acute tubular necrosis	12
Abruptio placenta-postpartum atony	Hysterectomy + hypogastric artery ligation	1000 mL/h	RBC: 9 FFP: 12 Platelet: 10 Fib: 16	-	10
Placenta percreta-uterine rupture	Hysterectomy + hypogastric artery ligation	1000 mL/h	RBC: 19 FFP: 20 Platelet: 24 Fib: 16	Maternal death	3
Postpartum atony	Hysterectomy + hypogastric artery ligation	750 mL/h	RBC: 8 FFP: 10 Platelet: 9 Fib: 16	-	6

RBC: Red blood cell, FFP: Fresh frozen plasma, Fib: Fibrinogen, WBC: White blood cell

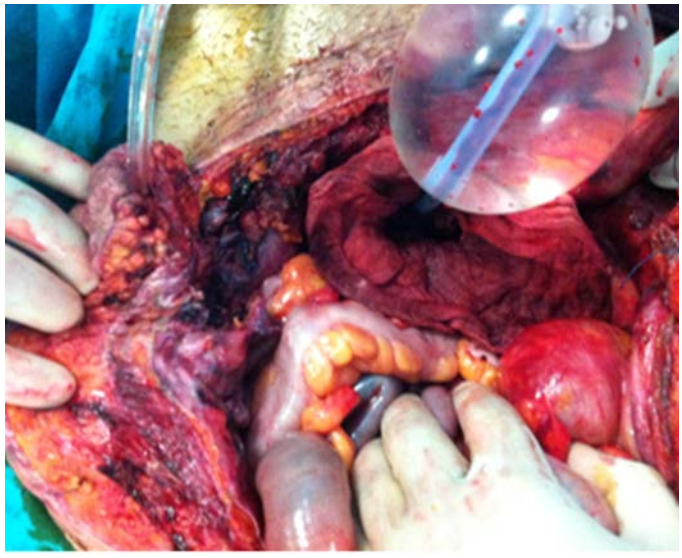

Figure 3. Bleeding stops in vascular structures and hemostasis is provided

Table 5. Laboratory values before vs after abdominal packing

	Preoperative	Postoperative
Hemoglobin, g/dL	4.86±0.7 (4-6)	7.33±1.21 (6-9)
Hematocrit	17±3 (12-22)	21.8±3 (19-27)
Platelet count, count/mm^3	66.500±26.000	116.000±38.000
INR	1.78±0.1 (1.6-2)	1.28±0.17 (1.1-1.6)
Fibrinogen	108±33.8 (70-150)	230±33.8 (198-260)

INR: International normalized ratio

Our last patient was brought to our emergency outpatient clinic directly, she was unconscious with fixed dilated pupils due to placenta percreta rupture at the 34th gestational week of pregnancy. Peripartum hysterectomy and bilateral iliac artery ligation were performed in this patient in an emergency laparotomy. Upon continuance of diffuse bleeding from the peritoneal surfaces and surgical site, Karateke packing was performed. Active bleeding was not observed through the drain in the intensive care unit postoperatively. However, the patient was diagnosed as having cerebrovascular hypoxia at the postoperative 3rd day and died of cardiac arrest on the same day. The clinical characteristics of these patients are presented in Tables 4 and 5.

Discussion

Pelvic packing techniques can be divided into 2 types: pads or roller gauze (sterile pads bound by suture threads or wrapped in a sterile bag) and balloon pack (Foley catheter or Bakri balloon). There is a difference in physical structure between these 2 types with practical consequences. First, it is easier and faster to assemble and apply the balloon pack because it is ready to be inflated and used immediately, but the pack formed by pads needs to be arranged and connected. Second, it is easier to adjust to match the size of the balloon packs to the size of the hemorrhagic areas with inflation or deflation of the balloon, but the addition or withdrawal of a pad from the constituted pack may be complicated[11]. Our packing method is important in regards to being the first to apply two methods in combination, as reported by Touhami et al.[7]. The strength of our study is that all of the patients referred to our clinic from external centers comprised "near miss" patients who require massive blood transfusion due to delayed surgical intervention. Classic abdominal packing is not sufficient to stop bleeding. The success of the treatment depends on the success of concurrent medical treatment, intensive care support therapy, and particularly the blood and blood product replacement protocols used. Karateke packing combines the methods defined by Naranjo-Gutiérrez et al.[10] and Charoenkwan K.[11]. Karateke packing is more efficient than the classic packing technique and is simpler and more easily applied compared with the method defined by Naranjo-Gutiérrez et al.[10]. In a study, packing was performed due to postpartum bleeding associated with uterine atony but hypogastric artery ligation was not performed in one of three patients in whom the Kittipat method was performed[11]. At the same time, a complicated clinical picture such as DIC did not accompany this case. Therefore, the efficiency of the method defined by Kittipat in patients developing DIC and requiring massive blood and blood product transfusions such as ours is controversial[11]. There is no consensus in the literature regarding the number of sponges required to provide hemostasis related with packing. If the sponges are not squeezed sufficiently, bleeding may continue and packing may result in failure. In the study performed by Deffieux et al.,[15] the authors failed to determine the required number of pads in a successful packing treatment. In Karateke packing, the compression strength exerted on the area of bleeding can be increased with the same number of sponges by increasing the traction force and volume of the intra-abdominal balloon. Thus, mechanical pressure applied to bleeding foci can be increased without having to perform relaparotomy to increase the number of sponges. Deffieux et al.[15] reported the success rate of abdominal packing as 62% in their study. Packing failed in 38% of cases and death occurred in 13[16]. In our series, hemostasis was provided with Karateke packing in all 6 patients including a patient in whom bleeding could not be controlled with classic abdominal packing. David Richardson et al.[16] reported that the mortality rate decreased by one-third when abdominal packing was performed in the early period after liver injury. However, the success rate of classic packing would be low in "near miss" patients who are referred in a complicated condition like in our patients. Besides that, Compartment syndrome, which is commonly mentioned in the literature when intra-abdominal pressure exceeds

the limit of 20 mm Hg, may develop and this is a serious complication that requires relaparotomy[17,18]. In our method, both abdominal fascial layers are not closed and decompression can be provided by releasing the distal tip of the Bakri balloon or by lowering the volume of the balloon when compartment syndrome develops. Symptoms of Compartment syndrome occurred in none of our patients. The depacking procedure is another important problem in abdominal packing. There is no consensus in the literature on this subject. However, it was reported that performing depacking after 24-48 hours reduced the recurrence rate of bleeding. Nicol et al.[19] reported that less bleeding occurred if depacking was performed after 24-48 hours; however, Caruso et al.[12] reported that risk for bleeding increased when sponges were removed earlier than 36 hours. Keeping sponges intra-abdominally for a long time may provide hemostasis but it can cause intra-abdominal adhesions and serious intra-abdominal infection. Abikhaled et al.[20] reported that the rate of abdominal abscess and death was lower when abdominal sponges were kept *in situ* less than 72 hours in 35 women with abdominal injuries. In our patients, the depacking procedure was performed when the bleeding through abdominal drain stopped and DIC laboratory findings regressed (international normalized ratio: <1.2, fibrinogen: >200, hemoglobin: >7). To prevent bleeding from the tissue while removing sponges adhered to visceral organs and surgical site, sponges should be soaked with a hot saline solution, ensured to be separated from the tissue, and removed with slow and gentle movements. The limitation of our method is the need for relaparotomy for the removal of the balloon and sponges.

Conclusion

Karateke abdominal packing is a very easy method to perform, more effective than the classic abdominal packing technique, with a low complication rate, and most importantly, life-saving in patients undergoing a peripartum hysterectomy due to PPH and thereafter experiencing diffuse hemorrhage.

Ethics

Ethics Committee Approval: The study was approved by the Zeynep Kamil Women and Children Research hospital local ethic committee (approval number 120-2015).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ç.K., Design: M.P., M.K., Data Collection or Processing: Ç.K., Ç.Y.A., E.B.E., Analysis or Interpretation: R.K., A.K., Literature Search: M.B.Ş., Writing: İ.Y., Ç.Y.A., Ç.K.

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

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The effect of first trimester hemoglobin levels on pregnancy outcomes

İlk trimester hemoglobin seviyelerinin gebelik sonuçlarına etkisi

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Abstract

Objective: The relationship between hemoglobin levels and pregnancy outcomes is still a challenging issue. There is a supported opinion about the increased adverse pregnancy outcomes both with low and high hemoglobin levels. In this study, we aimed to evaluate this association for first trimester hemoglobin levels in a Turkish population.

Materials and Methods: In this retrospective study, 1306 women who were followed up during their pregnancy and gave birth in our clinic were enrolled. The patients were divided into three groups: hemoglobin <11 g/dL (n=490), 11≤ hemoglobin <13 g/dL (n=673), and hemoglobin ≥13 g/dL (n=143). The hemoglobin <11 g/dL group was classified into two subgroups as hemoglobin ≤9 g/dL (n=64) and hemoglobin >9 g/dL (n=426). Demographic characteristics, first trimester hemoglobin levels, gestational age at delivery and mode, birth weight, Apgar scores, and pregnancy outcomes were recorded and compared between the groups.

Results: Pregnancy-induced hypertension, preterm birth, neonatal intensive care unit admission, birth weight, gestational age at delivery, Apgar scores, and postpartum hemorrhage were significantly different between the three groups. In the pairwise comparison, gestational age at delivery, birth weight, and first minute Apgar scores were higher in the 11≤ hemoglobin <13 g/dL group, and pregnancy-induced hypertension was more common in the hemoglobin ≥13 g/dL group as compared with the others. Moreover, the preterm delivery rate was highest in the hemoglobin ≥13 g/dL (26.6%) group and lowest (7.3%) in the 11≤ hemoglobin <13 g/dL group. The neonatal intensive care unit admission rate was higher both the hemoglobin <11 g/dL and hemoglobin ≥13 g/dL groups. Postpartum hemorrhage was more common in the hemoglobin <11 g/dL group as compared with the other groups. Furthermore, pregnancy-induced hypertension was more common in the hemoglobin ≤9 g/dL subgroup (p=0.012).

Conclusion: In conclusion, both low and high hemoglobin levels are related with adverse pregnancy outcomes. We suggest that hemoglobin levels must be screened during pregnancy to provide maternal and fetal well-being.

Keywords: First trimester, hemoglobin, pregnancy outcome

Öz

Amaç: Hemoglobin seviyeleri ve gebelik sonuçları arasındaki ilişki halen çelişkili bir konudur. Hem yüksek hem de düşük hemoglobin seviyelerinde artan olumsuz gebelik sonuçları olduğuna dair kanıtlar bulunmaktadır. Bu çalışmada, Türk popülasyonunda ilk trimester hemoglobin seviyesi ve gebelik sonuçları ilişkisi değerlendirilmiştir.

Gereç ve Yöntemler: Bu retrospektif çalışmaya gebelik takipleri ve doğumu kliniğimizde gerçekleştirilen 1306 hasta dahil edildi. Hastalar hemoglobin <11 g/dL (n=490), 11≤ hemoglobin <13 g/dL (n=673) ve hemoglobin ≥13 g/dL (n=143) olmak üzere 3 gruba ayrıldı. Hemoglobin <11 g/dL grubu da kendi içinde hemoglobin ≤9 g/dL (n=64) ve hemoglobin >9 g/dL (n=426) olmak üzere 2 alt gruba ayrıldı. Demografik özellikler, ilk trimester hemoglobin seviyeleri, doğum haftası, şekli ve kilosu, Apgar skorları ve gebelik sonuçları kaydedilerek gruplar arasında karşılaştırıldı.

Bulgular: Gebeliğin indüklediği hipertansiyon, preterm doğum, yenidoğan yoğun bakım ünitesi ihtiyacı, doğum kilosu, doğum haftası, Apgar skorları ve postpartum kanama sıklığı üç grup arasında anlamlı olarak farklı idi. İkili grup karşılaştırmasında, doğum haftası, doğum ağırlığı ve birinci dakika Apgar skorları 11≤ hemoglobin <13 g/dL grubunda daha yüksek iken, gebeliğin indüklediği hipertansiyon hemoglobin ≥13 g/dL grubunda diğer gruplardan daha sıktı. Ayrıca, preterm doğum hemoglobin ≥13 g/dL grubunda en yüksek (26,6%); 11≤ hemoglobin <13 g/dL grubunda en düşük (7,3%) oranda saptandı. Yenidoğan yoğun bakım ünitesi ihtiyacı hem hemoglobin <11 g/dL hem de hemoglobin ≥13 g/dL grubunda daha yüksekti. Postpartum kanama, hemoglobin <11 g/dL grubunda diğer gruplara oranla daha sıktı. Ayrıca, gebeliğin indüklediği hipertansiyon hemoglobin ≤9 g/dL alt grubunda daha sık olarak gözlendi (p=0,012).

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Sonuç: Sonuç olarak, hem düşük hem de yüksek hemoglobin seviyeleri olumsuz gebelik sonuçlarıyla ilişkilidir. Bu nedenle, maternal ve fetal iyilik halinin sağlanması için gebelikte hemoglobin seviyelerinin izlenmesi gerektiği kanaatindeyiz.
Anahtar Kelimeler: İlk trimester, hemoglobin, gebelik sonuçları

PRECIS: In this study, it is maintained that hemoglobin levels must be screened during pregnancy because both low and high hemoglobin levels are related with adverse pregnancy outcomes.

Introduction

Pregnancy has various effects on hematologic parameters. It is well known that hemoglobin (Hb) levels decrease during the first trimester, reaching minimum values in the late second trimester and tend to increase during the third trimester of pregnancy[1]. Therefore cut-off levels for determining anemia differ from healthy reproductive women. The World Health Organization (WHO) defines anemia as Hb levels <11.0 g/dL in the first and third trimesters and <10.5 g/dL in the second trimester in pregnant women[2]. Recently, there was a supported opinion about the relationship between anemia and adverse pregnancy outcomes. Several studies claimed that maternal anemia was risk factor for adverse pregnancy outcomes such as low birth weight (LBW), postpartum hemorrhage (PPH), cesarean section (CS), and preterm birth (PB)[3]. Unfortunately, there are conflicting results about this issue. In another study, it was demonstrated that moderate or severe anemia was related with small-for-gestational-age infants, and patients with mild anemia were found to have uneventful pregnancy outcomes. Moreover, it was reported that preterm delivery and LBW were not increased in women with Hb 8-10.9 g/dL[4]. Another interesting finding of the recent studies is the risk of adverse pregnancy outcomes in pregnancies with high Hb levels. In pregnancy, both total erythrocyte number and plasma volume increase, but Hb levels decrease according to the higher increment in plasma volume. This condition provides placental perfusion with reduced blood viscosity[5]. A high Hb concentration during pregnancy could result in placental infarcts due to the increased viscosity. As a consequence, these pregnancies can be complicated with pregnancy-induced hypertension (PIH), fetal growth restriction, and perinatal death[6]. The association between Hb levels and adverse pregnancy outcomes differs by trimesters. However, it is more evident in early pregnancy for low Hb levels, and it is evident in all trimesters for high Hb concentrations[7]. There are limited data about the relationship between adverse pregnancy outcomes and Hb levels in the first trimester of pregnancy in the Turkish population. In the present study, we evaluated the effect of first trimester Hb levels on pregnancy outcomes in our population.

Materials and Methods

This study was designed as a retrospective, observational, cross-sectional study. It was conducted in a university-affiliated training and research hospital between January 2016 and May 2017. Ethics committee approval is unnecessary for retrospective studies in our country. Our study complies with the Declaration of Helsinki.

A total of 1306 women who were followed up during their pregnancy and gave birth in our clinic were enrolled in the study. First, patients were divided into three groups: Hb <11 g/dL (n=490), 11≤ Hb <13 g/dL (n=673), and Hb ≥13 g/dL (n=143). Then, the Hb <11 g/dL group was classified into two subgroups as Hb ≤9 g/dL (n=64) and Hb >9 g/dL (n=426). The exclusion criteria for patient selection were determined as follows; unregular antenatal visits, lack of delivery data, absence of first trimester Hb values, age <16 and >40 years, multiple pregnancies, congenital malformations, pregnancies with history of diabetes mellitus, hepatic and renal failure, thyroid diseases, any uterine malformations, previous complicated pregnancy, alcohol and cigarette use, any prior placental abnormalities and PPH. Demographic characteristics of the study population such as age, gravida, parity, and gestational age at delivery and birth weight were recorded. Also, first trimester Hb levels were obtained from medical records. In our hospital, Hb levels are determined using a Coulter LH780 Analyzer (Beckman Coulter Ireland Inc, Mervue, Galway, Ireland). Pregnancy outcomes were compared between the groups. The main perinatal outcomes were accepted as stillbirth, gestational diabetes mellitus (GDM), PIH, PB, neonatal intensive care unit (NICU) admission, PPH, CS, and low Apgar scores, and these outcomes were obtained from hospital records. Stillbirth was judged as the death of a fetus during delivery[8]. GDM was established as if one or more of the followings were increased in the 75 g oral glucose tolerance test: fasting glucose ≥92 mg/dL, ≥180 mg/dL at 1 hour, and ≥153 mg/dL at 2 hours[9]. PIH was named as systolic blood pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg after the 20th gestational week, and PB was defined as births that occured between the 24-37th gestational weeks[10,11]. Neonates with shorter than 32 weeks of gestation, transient problems, cardiorespiratory monitoring requirement or presence of Respiratory Distress syndrome, severe jaundice, neonatal sepsis, and conditions requiring exchange transfusion were admitted to the NICU. PPH was defined as having a blood loss of ≥500 mL after vaginal delivery or ≥1000 mL after CS within 24 hours of delivery[12].

Statistical Analysis

Statistical analyses were performed using the Statistical

Package for the Social Sciences statistical software version 23.0 (SPSS, Chicago, IL). All data are reported as mean ± standard deviation, median [minimum (min), maximum (max)] values or in percentages. The Shapiro-Wilk test and probability plots were used to evaluate whether the variables followed normal distribution. The chi-square test and Fisher's exact test were performed to evaluate the relationship between categorical variables. According to the normality test results, the Mann-Whitney U test was used for continuous non-normally distributed variables, and the independent t-test was used for continuous normally distributed variables to compare the variables between two groups. For comparing more groups, the non-parametric Kruskal-Wallis test was performed, and the Bonferroni-Dunn procedure was used to compare statistically significant parameters between two groups. Moreover, for normally distrubuted variables, one-way ANOVA analysis was performed to compare the variables between more than two groups. A p value of ≤0.05 was determined as statistically significant.

Results

The mean age of all study participants was 27.24±6.21 years. The median gravida was 2 (min=1, max=10) and parity was 1 (min=0, max=8). The mean gestational age at delivery was 38.16±2.37 weeks and the mean birth weight was 3134.78±600.96 grams. The demographic data and pregnancy outcomes of three main groups were compared and are presented in Table 1. PIH, PB, NICU admission, birth weight, gestational age at delivery, Apgar scores, and PPH were significantly different between the three groups. In the pairwise comparison; gestational age at delivery, birth weight, and first-minute Apgar scores were significantly higher in 11≤ Hb <13 g/dL group, and PIH was more common in the Hb ≥13 g/dL group as compared with the other groups. Moreover, the PB rate was highest in the Hb ≥13 g/dL (26.6%) group and was lowest (7.3%) in the 11≤ Hb <13 g/dL group. The NICU admission rate was significantly higher in both the Hb <11 g/dL and Hb ≥13 g/dL group as compared with the 11≤ Hb <13 g/dL group. Furthermore, PPH was significantly more common in the Hb <11 g/dL group as compared with the other groups. The comparison of demographic characteristics and pregnancy outcomes between the Hb ≤9 g/dL and 9< Hb <11 g/dL group is shown in Table 2. There was no difference between the two groups according to gravida, parity, age, gestational age at delivery, birth weight, Apgar scores, stillbirth, GDM, PB, CS, NICU admission, and PPH rates. Contrary to these, PIH was significantly higher in the Hb ≤9 g/dL group (p=0.012).

Discussion

The main findings of the study were as follows: PIH was more common in the high Hb group, and the incidences of PB and NICU admission were higher both in the high and low Hb groups. Moreover, PPH was common in the low Hb group. Gestational age at delivery, birth weight, and first-minute Apgar scores were significantly higher in the 11≤ Hb <13 g/dL group, and there was no difference according to pregnancy outcomes between the very low and low Hb groups, except PIH. During pregnancy, many hormonal changes occur to provide adequate blood flow from the maternal to the fetal

Table 1. Demographic characteristics and pregnancy outcomes of all study groups

	Hb <11 g/dL (n=490)	11≤ Hb <13 g/dL (n=673)	Hb ≥13 g/dL (n=143)	p
Age (years)	26.97±6.07	27.45±6.33	27.17±6.12	0.410
Gravida (n)	2 (1-8)	2 (1-10)	2 (1-9)	0.887
Parity (n)	1 (0-5)	1 (0-7)	1 (0-8)	0.465
Gestational age at delivery (week)	37.78±2.62	38.6±1.95	37.43±2.8	<0.001
Birth weight (grams)	2992.8±635.1	3269.2±512.7	2988.3±713.3	<0.001
Stillbirth (n, %)	8 (1.6%)	7 (1.0%)	3 (2.1%)	0.511
GDM (n, %)	27 (5.5%)	35 (5.2%)	8 (5.6%)	0.965
PIH (n, %)	31 (6.3%)	23 (3.4%)	21 (14.7%)	<0.001
Preterm birth (n, %)	78 (15.9%)	49 (7.3%)	38 (26.6%)	<0.001
Cesarean section (n, %)	209 (42.7%)	259 (38.5%)	70 (49.0%)	0.051
NICU admission (n, %)	60 (12.2%)	36 (5.3%)	25 (17.5%)	<0.001
Apgar 1st min	8.69±1.37	8.83±1.04	8.65±1.44	0.005
Apgar 5th min	9.67±1.4	9.81±1.08	9.62±1.58	0.012
PPH (n, %)	63 (12.9%)	37 (5.5%)	13 (9.1%)	<0.001

GDM: Gestational diabetes mellitus, Hb: Hemoglobin, NICU: Neonatal intensive care unit, PIH: Pregnancy-induced hypertension, PPH: Postpartum hemorrhage

Table 2. Demographic characteristics and pregnancy outcomes of Hb ≤9 g/dL and 9< Hb <11 g/dL groups

	Hb ≤9 g/dL (n=64)	9< Hb <11 g/dL (n=426)	p
Age (years)	28.14±7.16	26.79±5.88	0.154
Gravida (n)	2 (1-7)	2 (1-8)	0.600
Parity (n)	1 (0-5)	1 (0-4)	0.832
Gestational age at delivery (week)	37.56±2.51	37.81±2.64	0.291
Birth weight (grams)	2958.5±617.3	2997.9±638.2	0.641
Stillbirth (n, %)	2 (3.1%)	6 (1.4%)	0.281
GDM (n, %)	3 (4.7%)	24 (5.6%)	0.999
PIH (n, %)	9 (14.1%)	22 (5.2%)	0.012
Preterm birth (n, %)	13 (20.3%)	65 (15.3%)	0.303
Cesarean section (n, %)	29 (45.3%)	180 (42.3%)	0.645
NICU admission (n, %)	10 (15.6%)	50 (11.7%)	0.376
Apgar 1st min	8.61±1.66	8.7±1.32	0.948
Apgar 5th min	9.53±1.82	9.69±1.33	0.771
PPH (n,%)	8 (12.5%)	55 (12.9%)	0.927

GDM: Gestational diabetes mellitus, Hb: Hemoglobin, NICU: Neonatal intensive care unit, PIH: Pregnancy induced hypertension, PPH: Postpartum hemorrhage

unit. One of these changes is increased plasma renin and decreased atrial natriuretic peptide levels. Also, erytropoetin secretion tends to increase and results in a rise in red blood cell mass. On the other hand, plasma volume expands nearly 50% and consequently, Hb levels decrease. Disturbance of these mechanism leads to hemoconcentration and high Hb levels[13,14]. PIH is still an important cause of maternal and fetal mortality and morbidity. Although the underlying mechanism has not been fully elucidated, recent studies have shown that increased Hb levels leading to vasoconstriction is one of the mechanisms of PIH[15]. Also, the loss of protein and increment in vascular permeability causes a decrement in intravascular volume and high Hb concentrations in preeclampsia[16]. In a study by Pritchard et al.,[17] the average hematocrit was higher in preeclampsia as compared with healthy pregnant women. In other studies, a significant relationship between high first-trimester Hb levels and preeclampsia was demonstrated[18,19]. Similarly, in this present study, PIH was more common with high Hb levels. We and others suggest that the changes of hematologic changes in PIH start early in the first trimester and monitoring Hb levels could be used to follow up pregnancies at high risk for uteroplacental insufficiency.[20] Recent studies evaluating the PB risk in pregnant women in relation to Hb levels had conflicting results. Scanlon et al.,[13] who divided the patient group into 7 levels as very low, low, low-normal, normal (reference group), high-normal, high, and very high Hb groups showed that patients with a first-trimester Hb concentration below the reference range had an elevated risk of PTB. Furthermore, in a study of a Chinese population, elevated PB risk was found in the low first-trimester Hb group[21,22]. On the other hand, no relationship was found between PB and first-trimester Hb levels in the study of Hamalainen et al.[23]. For high Hb levels, Zhang et al.[22] found reduced risk for PB, and other studies claimed that no association was present between PB and high Hb levels.[13,24] Zhou et al.[25] reported slightly increased risk for PB with high Hb levels. In this present study, PB rates were higher with both low and high Hb levels. Moreover, NICU admission rates were higher in both the high and low Hb groups, which could be related to prematurity and accompanying conditions. PH is one of the leading causes of maternal mortality. Oxygen and Hb transportation is the cornerstone of uterine contractions and it is claimed in the literature that anemic patients were more likely to experience uterine atony due to the absence of these mechanisms[26,27]. In the study of Sehgal et al.,[4] pregnant women with mild-to-moderate anemia were found to have more PPH. Similar to their study, we demonstrated that patients with Hb <11 g/dL had more PPH as compared with those in the 11≤ Hb <13 g/dL and Hb ≥13 g/dL groups. There is no consensus about iron replacement, to whom and how many milligrams should be given in pregnancy. The WHO recommends 30-60 mg daily iron during pregnancy. These data were based on the reduced risk of LBW with daily iron supplementation, increased risk for adverse effects, and adverse pregnancy outcomes for high Hb levels[28,29]. Supporting these recommendations, we found higher birth weights and Apgar scores, and lower PB in the 11≤ Hb <13 g/dL group and we suggest that appropriate

Hb levels with iron supplementation must be constituted to provide maternal and fetal well-being. However, we did not investigate the effects of iron supplementation on our pregnancy outcomes, which is one of the major limitations of our study. Another interesting finding of our study was that there was no difference with regard to pregnancy outcomes between the very low and low Hb groups, except PIH. A few studies that investigated the effects of severe or moderate anemia demonstrated that patients with moderate and severe anemia were more prone to uterine atony and PPH[26,27]. Pregnant women with mild anemia are generally expected to have uneventful pregnancies if well managed with iron supplementation[4]. Another study showed that Hb 8-10.9 g/dL was not associated with an increased PB and LBW risk[22,30]. We suggest that our non-significant results between the very low and low Hb groups might be related to the small patient population with severe anemia.

Study Limitation

This study has several limitations. First, we did not investigate the effects of iron supplementation on our pregnancy outcomes. Second, we had small patient population with very low Hb levels to compare the pregnancy outcomes between low and very low Hb levels. Lastly, we only evaluated the first trimester Hb levels and it might be more appropriate to clarify the relationship between all trimester Hb levels and pregnancy outcomes.

Conclusion

In conclusion, both low and high Hb levels are related with adverse pregnancy outcomes. To provide maternal and fetal well-being, we must routinely screen the first trimester Hb levels and think about supplementing iron if it is appropriate.

Ethics

Ethics Committiee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.D.Ç., E.Ü., Concept: B.D.Ç., Design: Ü.A.T., B.D.Ç., Data Collection or Processing: Ü.A.T., M.A., S.Ö., Analysis or Interpretation: M.A., S.Ö., E.Ü., Literature Search: B.D.Ç., S.Ö., Writing: B.D.Ç., E.Ü.
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Early-onset neonatal infection in pregnancies with prelabor rupture of membranes in Kosovo: A major challenge

Kosova'da erken membran rüptürü olan gebelerde erken başlangıçlı neonatal enfeksiyon: Önemli bir sorun

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Abstract

Objective: Prelabor rupture of membranes (PROM) is a common event in obstetrics that has a major impact in pregnancy outcome. This condition is linked to a number of pregnancy and birth complications with early-onset neonatal infection (EONI) being one of the major threats. This study was undertaken to determine the rate of neonatal infection in newborn infants with a maternal history of PROM and to evaluate the association of risk factors with neonatal infection following PROM.

Materials and Methods: A cross-sectional descriptive design was used to analyze a population of 200 pregnant women presenting to the Obstetrics and Gynecology Tertiary Center in Kosovo (between 2013 and 2015) with PROM who gave birth to single newborns. Data including demographic characteristics, neonatal outcome, and risk factors for infectious neonatal morbidity were recorded and analyzed.

Results: The study included 200 pregnant women with PROM and their newborns. Participant demographics included: the majority were young, aged between 20 and 29 years (67%), primiparous (67.5%), unemployed (92%), completed secondary level of education (83%), and with middle socioeconomic status (86%). Overall, 13% of the newborns had early-onset neonatal infection, and sepsis was proven in 5% of cases. Newborns of mothers with risk factors such as preterm (<37 weeks) PROM, low gestational weight at birth, prolonged rupture of membranes, maternal colonization, and low Appearance, Pulse, Grimace, Activity, Respiration score at birth had higher rates of infection compared with newborns of mothers without these risk factors.

Conclusion: The rate of EONI in pregnancies complicated with PROM continues to be a global challenge in perinatology, and as this study reports, also a major challenge for Kosovo. Future research, revision and improvement on prenatal care and practices, timing of delivery, medical treatment, and prophylactic use of antibiotics in PROM are needed to reduce rates.

Keywords: Prelabor rupture of membranes, early-onset neonatal infection, risk factors

Öz

Amaç: Obstetride erken membran rüptürü (EMR), gebelik sonuçlarında önemli bir etkiye sahip olan yaygın bir durumdur. Bu durum bir dizi gebelik ve doğum komplikasyonu ile bağlantılıdır ve erken başlangıçlı neonatal enfeksiyon başlıca tehditlerden biridir. Bu çalışmada, maternal EMR öyküsü olan yenidoğan infantlarda neonatal enfeksiyon oranının belirlenmesi ve EMR'yi takiben neonatal enfeksiyon ile risk faktörleri ilişkisinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler Kosova'da 3. Basamak Kadın Doğum ve Jinekoloji Merkezi'ne EMR ile başvuran (2013-2015 yılları arasında) ve tek yenidoğan dünyaya getiren 200 gebe kadın populasyonunu analiz etmek için kesitsel tanımlayıcı tasarım kullanıldı. Demografik özellikleri, neonatal sonuçları ve enfeksiyöz neonatal morbidite için risk faktörlerini içeren veriler kayıt ve analiz edildi.

PRECIS: The purpose of this study was to determine the rate of neonatal infection in newborn infants with a maternal history of prelabor rupture of membranes and to evaluate association of risk factors with neonatal infection in pregnancies complicated with prelabor rupture of membranes.

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Bulgular: Çalışmaya, EMR'si olan 200 gebe kadın ve yenidoğanları dâhil edildi. Katılımcıların demografik özellikleri incelendiğinde; çoğunluğunun genç, yaş aralığının 20-29 (%67), primipar (%67,5), işsiz (%92), ortaöğretim düzeyinde eğitim tamamlamış (%83) ve orta sosyoekonomik statüde (%86) olduğu saptandı. Genel olarak, yenidoğanların %13'ünde erken başlangıçlı neonatal enfeksiyon mevcuttu ve olguların %5'inde sepsis varlığı kanıtlandı. Preterm EMR (<37 hafta), doğumda düşük gestasyonel ağırlık, uzamış membran rüptürü, maternal kolonizasyon ve doğumda düşük APGAR skoru gibi risk faktörleri olan annelerin yenidoğanları, bu risk faktörleri olmayan annelerin yenidoğanları ile karşılaştırıldığında daha yüksek enfeksiyon oranına sahipti.
Sonuç: EMR ile komplike gebeliklerde erken başlangıçlı neonatal enfeksiyon oranı, perinatolojide küresel bir sorun olmaya devam etmektedir ve bu çalışmanın bildirdiği gibi Kosova için de önemli bir sorundur. Oranların azaltılması için; daha ileri araştırmalar, prenatal bakım ve uygulamaların revizyonu ve geliştirilmesi, doğru doğum zamanlaması, EMR'de profilaktik antibiyotik kullanımı ve medikal tedavi gerekmektedir.
Anahtar Kelimeler: Erken membran rüptürü, erken başlangıçlı neonatal enfeksiyon, risk faktörleri

Introduction

Kosovo continues to have a high rate of early neonatal morbidity and mortality[1]. According to Azemi et al.[2] and local annual perinatal reports, perinatal infections are one of the three main causes of early neonatal morbidity and mortality. There are, however, limited data about the rate and risk factors of early-onset neonatal infection (EONI) in pregnancies, including the focus of this study, EONI in pregnancies complicated with prelabor rupture of membranes (PROM), in Kosovo. PROM refers to rupture of the chorioamniotic membranes prior to the onset of labor and prior to the onset of clinically-apparent labor contractions. It can occur at any gestational age, thus this condition has been classified as "preterm PROM" or "term PROM", depending on whether the event occurs before or after 37 weeks of gestation[3]. Early-onset infectious morbidities in newborn implies infections that occur from birth to seven completed days after birth, and these are considered to be one of the major threats in patients with PROM. The time interval between the rupture of membranes and onset of labor pain is called the latent period, and the time interval between the rupture of membranes and delivery is called the interval period (IP). The minimum latency for diagnosis of PROM is one hour. According to Caughey et al.,[4] latency after membrane rupture is inversely correlated with gestational age at membrane rupture. At term, 50% of pregnancies complicated with PROM will go into labor spontaneously within 12 hours, 70% within 24 hours, 85% within 48 hours, and 95% within 72 hours. Among pregnancies complicated with preterm PROM, 50% will go into labor within 24 to 48 hours, and 70-90% within seven days.[4] PROM is a common obstetric complication, but it is difficult to give an accurate incidence because of the wide variations reported in the existing literature. Recent data suggest that PROM occurs in approximately 10 to 12% of all pregnancies. Of these pregnancies, PROM complicates about 8% of term pregnancies and 2 to 3% of preterm pregnancies.[4] A greater incidence of PROM was found in earlier research studies, which narrowed the PROM incidence range as 14 to 17%[5]. The etiology of PROM is almost certainly multi-factorial and in some cases unknown. The biologic mechanisms behind the development of PROM include intrinsic membrane weakness, mechanical stress, and ascending infection.[6] PROM is linked to a number of adverse maternal and neonatal outcomes. The most frequent maternal consequences associated with PROM are chorioamnionitis, endomyometritis, wound infection, pelvic abscess, bacteremia, and postpartum hemorrhage. One of the most serious neonatal consequences associated with PROM is EONI. Physicians, and especially obstetricians, have long debated whether intrauterine infection is a cause or consequence of PROM and it seems likely both pathways are possible. The case for intrauterine infection being a consequence of PROM appears to be proved. Prior to rupture of membranes, the amniotic cavity is nearly always sterile. The physical properties of the intact placental membranes usually represent an effective barrier in preventing entry of bacteria. With rupture of membranes, protection of the fetus from the external micro-organisms ceases, bacteria from the lower genital tract typically enter the amniotic cavity, thus increasing the potential for subsequent maternal, fetal, and neonatal infection.[7] To determine the rate of neonatal infection in newborn infants with a maternal history of PROM, and to evaluate the association between risk factors and neonatal infectious morbidity.

Materials and Methods

This cross sectional descriptive study was conducted at the Tertiary Obstetrics and Gynecology Clinic University Clinical Center of Kosovo from September 2013 to July 2015. The study participants included pregnant women who were admitted with PROM. The selection of the study participants was based on the defined inclusive criteria: women with singleton pregnancy at or >28 weeks of gestation, presenting with PROM, and giving birth within 72 hours after PROM. Women with preexisting comorbidities and fetal anomalies were excluded from the study. A total of 200 pregnant women who presented with PROM who fulfilled the study's inclusion/exclusion criteria and their newborns were included in the study informed consent was obtained from all potential participants. This study was approved by Research Ethics Committee of the Prishtina University, University Clinical Center of Kosovo (approval number 1451). A specific questionnaire and evaluation form was prepared and used to collect data prospectively at admission and thereafter. Data covering demographic characteristics and clinical data including gestational age at birth, gestational

weight at birth, 1st and 5th minute Apgar (Appearance, Pulse, Grimace, Activity, Respiration) score, interval from PROM to delivery, maternal colonization, and early neonatal infectious morbidity were recorded and analyzed. Confirmation of gestational age was based on the last menstrual period, and in patients with irregular cycle or unknown last menstrual period, the gestational age was determined based on the medical records of the first trimester ultrasound examination. Confirmation of the diagnosis of rupture of membranes was documented through sterile speculum examination confirming the pooling of amniotic fluid in the posterior vaginal fornix or/and direct visualization of fluid leakage from the cervical canal. An ultrasound examination was performed to confirm fetal wellbeing. Immediately after delivery, the physical condition of the newborn was evaluated using Apgar scores by a neonatologist who was present at the birth. The newborns were observed during the first seven days of life during the early neonatal period. EONI was the main outcome registered and studied. The occurrence of early neonatal infection was diagnosed by a neonatologist. Diagnosis of the EONI was made using a combination of the clinical signs of infection (hypothermia, respiratory distress, lethargy, hypotonia, irritability, bradycardia) with laboratory findings (total blood count with differential, CRP ≥10 mg/dL) or by positive culture of the urine, cerebral fluid or blood. With the purpose of determining differences in demographic characteristics, the participants were divided in two groups: mothers of newborns without infection (group 1) and mothers of newborns with infection (group 2). With the aim of determining the association of risk factors and neonatal infection, all newborns were divided into two groups based on the presence of the neonatal infection: newborns without infection (group 1) and newborns with infection (group 2).

Statistical Analysis

aSPSS 17.0 statistical software was used for statistical analysis. Pearson's chi-square test and the t-test were used as appropriate. Clinical characteristics were compared using descriptive statistics and are presented as percentages, frequencies, and means.

Results

Two hundred pregnant women complicated with PROM and their newborns were included in the study. The mean age of patients was 27.5 years [standard deviation (SD) ±5.5]. The majority (67%) of the participants were young, aged 20-29 years, primiparous (67.5%), unemployed (82%), had completed secondary level education (44.5%), with middle socioeconomic status (66.5%), and the average number of people per household was revealed as 6.3 (SD±3.7). Although there were no statistically significant differences regarding mean age, family size, and socio-economic status between the two groups, the registered percentage difference regarding parity and employment status was statistically significant ($p<0.05$) (Table 1). Twenty-six (13%) of the 200 newborns were diagnosed as having EONI. Of the 26 infants with neonatal infection, the highest percentage had pneumonia (46.2%), followed by sepsis (38.5%), systemic inflammatory response syndrome (11.5%), and 1 infant had meningitis. Exitus letalis was registered in two infants (1%). These were newborns of mothers with PROM in less than 37 weeks of gestation and who were found to have neonatal sepsis. The average weight of newborns with regard to

Table 1. Comparison of maternal characteristics

Variable	Total n=200	Mothers of neonates without infection n=174	Mothers of neonates with EONI n=26	p
Mean age ± SD[a,b]	27.5±5.0	27.4±5.4	27.8±5.8	0.758
Parity [a]				
Nulliparous	135 (67.5)	124 (71.3)	15 (57.7)	0.003
Multiparous	65 (23.5)	50 (28.7)	11 (42.3)	
Level of education [a]				
Elementary	50 (25.0)	43 (24.7)	7 (26.9)	0.178
Secondary	89 (44.5)	74 (42.5)	15 (57.7)	
University	61 (30.5)	57 (32.8)	4 (15.4)	
Employment status[a]				
Yes	35 (17.5)	34 (19.5)	1 (3.8)	0.049
No	165 (82.5)	140 (80.5)	25 (96.2)	
Socioeconomic status[a]				
Low	18 (9.0)	14 (8.0)	4 (15.4)	0.301
Medium	133 (66.5)	115 (66.1)	18 (69.2)	
High	49 (24.5)	45 (25.9)	4 (15.4)	
Family size/ ± SD[a,b]	6.3±3.7	6.2±3.8	6.9±3.3	0.368

[a]Values are expressed as number (percentage) or mean ± SD, SD: Standard deviation
[b]Fisher exact test and t test with statistical significance at $p<0.05$ EONI: Early-onset neonatal

Table 2. Risk factors and neonatal infection

Variable	Group 1 newborns without infection (n=174)	Group 2 newborns with EONI (n=26)	p
Gestational age[a]			
≥37 weeks, n (%)	137 (78.7)	7 (26.9)	<0.01
<37 weeks, n (%)	37 (21.2)	19 (73.1)	
Gestational weight at birth (g)[a,b] Mean ± SD	3183.2±567.2	2466.2±853.7	<0.01
Interval period (hours)[a,b] Mean ± SD	23.3±12.6	34.7±20.3	<0.01
Maternal colonization[a]			
Positive, n (%)	40 (22.9)	21 (80.7)	<0.01
Negative, n (%)	134 (77.0)	5 (19.2)	
Mode of delivery[a]	57 (32.8)	12 (46.2)	>0.49
Vaginal spontaneous, n (%)	70 (40.2)	7 (26.9)	
Vaginal induced, n (%)	46 (26.4)	7 (26.9)	
CS, n (%)	1 (0.6)		
VE, n (%)			
Apgar score, mean ± SD[a,b]			
1st minute	7.3±1.53	5.7±0.99	<0.01
5th minute	8.4±1.57	7.0±0.86	<0.01

[a]Values are expressed as number (percentage) or mean ± SD, [b]Fisher exact test and t test with statistical significance at p<0.05, CS: Cesarean section, VE: Vacuum extraction, Apgar score: Appearance, Pulse, Grimace, Activity, Respiration, EONI: Early-onset neonatal

registered neonatal infection was 2466.9±853.7 g, whereas in those without neonatal infection it was 3183.2±567.2 g. The difference between the average values was statistically significant (p<0.001). The mean interval from PROM to delivery in patients with registered neonatal infection was 34.7±20.3 hours, and the mean interval in the group without neonatal infection was 23.3±12.6 hours (Table 2). According to t-test the difference was statistically significant (p<0.001). The data analysis of the high vaginal swab results revealed that out of 61 colonized mothers, 21 had newborns with infection, thus indicating a significant statistical association between maternal genital tract colonization and EONI. The difference between the mean Apgar scores at the 1st and 5th minutes after birth between the groups with and without neonatal infection was statistically significant (p<0.05). There were no significant differences according to the mode of delivery between the groups (Table 2).

Discussion

EONI remains one of the most serious complications in pregnancies complicated with PROM and poses a major health challenge, especially for developing countries with higher reported rates of early neonatal morbidity and mortality. In this study, out of 200 newborns born following PROM, 13% had EONI. The incidence found in this study is comparable with the results of other studies found in the literature; however, it is important to acknowledge that the literature reveals a wide variation of EONI incidence in pregnancies complicated with PROM.

The findings in this study are in congruence with Asindi et al.[8] who reported an incidence of 14% of neonatal infection after PROM. In contrast, Popowski et al.,[9] in a study among 399 pregnant women complicated with PROM, reported that 4.3% of newborns were diagnosed as having EONI. In a cohort study conducted in China, Wu et al.[10] reported a much greater incidence (25%) of EONI in pregnancies complicated with PROM. According to our study, the observed rate of EONI in pregnancies complicated with PROM indicates a high neonatal infectious morbidity rate compared with the results of more developed countries. Out of all 200 newborns, early-onset neonatal sepsis was proven in 5% of cases. This finding is in agreement with Alam et al.[11] who conducted a cohort study over a five-year period. Authors analyzed neonatal outcome among neonates who had a maternal history of PROM and they reported an incidence of early neonatal sepsis as 4%. In another prospective study among 135 infants born after PROM, the reported incidence of early-onset neonatal sepsis was 8.1%.[12] Similarly, Lee at al.[13] reported an incidence of 6.5% of culture-proven sepsis among neonates born from pregnancies complicated with PROM. This greater incidence of sepsis among infants born after PROM might be due to the fact that these previous studies enrolled newborns born after PROM with a latency period of greater than 24 hours, whereas in our research we included newborns born after PROM for whom the duration of latency period was restricted to a minimum of 1 hour and maximum of 72 hours. In this study, the majority (73%) of infected neonates were born preterm. This higher rate of EONI among preterm infants is expected

and is explained by prematurity and associated incomplete maturation of the immune system, which increases the likelihood of infections. The association of birthweight and neonatal outcome is well known. In a recent study, the median birthweight of a group of neonates with EONI was lower (2446 g) than of neonates without infection (3183 g), the difference being statistically significant ($p<0.05$). This higher rate of EONI among newborns with lower birth weight is expected and is explained by the immaturity of systems of organs including the immune system. This is in accordance with the finding that birth weight is a significant predictor of neonatal outcome and it is inversely related to risk of EONI, as observed in other studies.[14]

The interval between PROM and delivery is a factor that may influence maternal and fetal wellbeing; prolongation of the interval from PROM to delivery increases the incidence infection.[15] In recent research, the interval from membrane rupture to delivery ranged from 7 to 70 hours with a mean of 24.8 hours. Our evaluation of PROM to delivery interval in terms of neonatal infection showed a significantly longer IP of 34.7 hours in the group with neonatal infection versus 23.3 hours in the group without neonatal infection. This finding is in accordance with the findings of Herbst and Kallen[16]. They reported that the duration of membrane rupture was an independent risk factor for neonatal sepsis and this risk of neonatal sepsis increased independently and nearly linearly with duration of membrane rupture up to 36 hours, with an odds ratio of 1.29 for each 6-hour increase in membrane rupture duration.

A number of studies have highlighted the association between neonatal infection in the first days of life and maternal genital tract colonization. Among the 200 participants included in the present article, high vaginal swab results were positive in 61 (30.5%) cases. Data analysis revealed that out of 61 colonized mothers, 21 had newborns with infection, thus indicating a significant statistical association between maternal genital tract colonization and EONI. Evaluation of the 1st and 5th minute Apgar scores among neonates with and without neonatal infection showed that Apgar scores were statistically significantly lower in the group with neonatal infection compared with group without neonatal infection ($p<0.05$). Analysis in a recent study identified 1st minute Apgar scores of 5.7±1.5 as a strong risk factor for the development of early neonatal infection, thus this finding supports observation and screening of neonates for possible EONI. In accordance with our results, Hayun et al.[17] reported that Apgar score, gestational age, and weight at birth are risk factors for EONI. In summary, the data reported in the present study are in alignment with the literature from developing countries, demonstrating high neonatal infectious morbidity rates in pregnancies complicated with PROM.

Study Limitations

The limitation of the present study is its sample size. Future research using larger sample sizes are warranted to replicate these research findings.

Conclusion

The finding of an EONI rate of 13%, of which 5% were confirmed as early-onset neonatal sepsis, provides additional evidence indicating a high level of EONI among newborns of mothers complicated with PROM.

Risk factors for the development of EONI in pregnancies complicated with PROM are: PROM-delivery interval, low gestational weight and low gestational age at birth, maternal colonization, and low Apgar score. The findings of this study contribute to the larger literature by confirming that neonatal infection in pregnancies complicated with PROM is present as a challenge in Kosovo. Future steps to be undertaken include revision and improvement of antenatal care practices and prophylactic use of antibiotics in PROM.

Ethics

Ethics Committee Approval: This study was approved by Research Ethics Committee of the Prishtina University, University Clinical Center of Kosovo (approval number 1451).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: V.A.I., Concept: V.A.I., Design: V.A.I., A.E.M., Data Collection or Processing: V.A.I., Analysis or Interpretation: R.I., V.A.I., Literature Search: V.A.I., R.I., A.E.M., Writing: V.A.I.

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YKL-40 in the diagnosis, prediction of prognosis, and platinum sensitivity in serous epithelial ovarian cancer

Seröz epitelyal over kanserinde tanıda, prognoz ve platin duyarlılığı öngörüsünde YKL-40 kullanımı

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Abstract

Objective: To evaluate the use of YKL-40 in the discrimination between benign and malignant adnexal mass and to determine its prognostic value in assessing residual tumor after primary cytoreduction and platinum sensitivity in serous epithelial ovarian carcinoma (EOC).

Materials and Methods: During the three years from January 2015 to December 2017, a nonconsecutive series of 100 patient (60 malignant, 40 benign) who underwent surgery for an adnexal mass were enrolled in the study. Preoperatively, serum samples were collected for YKL-40 level analysis.

Results: YKL-40 [receiver operator characteristics (ROC)-area under curve (AUC)=0.83] was a significantly better predictor of EOC than cancer antigen-125 (ROC-AUC=0.75). Using a cut-off for YKL-40 of 47.7 ng/mL had a sensitivity of 80% and a specificity of 70%. Higher serum YKL-40 levels were associated with advanced stage, higher grade, residual tumor after primary cytoreduction and recurrence. Platinum-sensitive patients had significantly elevated levels of YKL-40 compared with platinum-resistant or refractory patients.

Conclusion: The results obtained from our study support the use of serum YKL-40 for the discrimination between malignant and benign ovarian tumors. YKL-40 levels in patients with serous EOC may also predict disease residual disease after primary cytoreduction and recurrence. Further studies are needed to understand the relationship between YKL-40 and platinum sensitivity.

Keywords: YKL-40, ovarian cancer, biomarker, platinum sensitivity

Öz

Amaç: Benign ve malign adneksiyel kitlelerin ayrımında YKL-40 kullanımını araştırmak ve YKL-40'ın seröz epitelyal over kanserinde (EOK) primer sitoredüksiyon sonrası rezidual tümörü ve platin sensitivitesini öngörmedeki etkinliğini değerlendirmek.

Gereç ve Yöntemler: Ocak 2015-Aralık 2017 arasındaki üç yıl boyunca, adneksiyel kitle nedeniyle opere olan, ardışık olmayan 100 hasta (60 malign, 40 benign) çalışmaya dahil edildi. Preoperatif dönemde, serum örnekleri YKL-40 analizi için toplandı.

Bulgular: YKL-40 [alıcı işlem karakteristikleri (AİK)-eğri altında kalan alan (EAKA)=0,83], EOK'yi kanser antijen-125'e (AİK-EAKA=0,75) göre anlamlı olarak daha iyi predikte etmektedir. YKL-40 için 47,7 ng/mL eşik değeri kullanıldığında, %80 sensitivite ve %70 spesifisite sağlanmaktadır. Yüksek serum YKL-40 değerleri, ileri evre, ileri derece, primer sitoredüksiyon sonrası rezidual tümör ve rekürens ile ilişkiliydi. Platin sensitif hastalarda, platin dirençli ve platin refrakter hastalara göre anlamlı olarak yükselmiş YKL-40 seviyeleri mevcuttu.

Sonuç: Çalışmamızdan elde edilen bulgular, malign ve benign over tümörlerinin ayrımında serum YKL-40'ın kullanılmasını desteklemektedir. Seröz EOK'li hastalarda YKL-40 seviyeleri, primer sitoredüksiyon sonrası reizdual hastalığı ve rekürensi predikte edebilir. YKL-40 ile platin sensitivtesi arasındaki ilişkinin anlaşılabilmesi için, gelecek çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: YKL-40, over kanseri, biyomarker, platin sensitivitesi

PRECIS: YKL-40 may be used in the management of adnexal masses and may predict residual disease after primary cytoreduction and recurrence.

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Introduction

Ovarian cancer is a common gynecologic malignancy associated with poor prognosis worldwide. Patients with early stage ovarian cancer have a 5-year overall survival of 70-90%. However, more than 70% of women are diagnosed as having stage III or IV disease, which has a 5-year survival rate of around only 25%[1]. This poor prognosis emphasizes the need for early detection in screening programs and prognostic markers to guide individual cancer treatment. The standard treatment of early epithelial ovarian carcinoma (EOC) is full staging including pelvic and paraaortic lymphadenectomy. Debulking surgery followed by platinum-based chemotherapy is the cornerstone of treatment of advanced EOC[2]. Neoadjuvant chemotherapy followed by interval cytoreduction has been a debatable alternative option. A metaanalysis of 21 non-randomized trials concluded that survival between two groups was similar[3]. Subsequently, Vergote et al.[4] performed a randomized trial including patients with stage IIIC-IV EOC and found that optimal cytoreduction was higher in an interval cytoreduction group compared with a primary cytoreduction group with significantly less morbidity in the interval cytoreduction group. However, a number of studies showed that leaving no residual tumor following primary debulking surgery was the single most important independent prognostic factor in advanced EOC[4,5]. Patients who are at higher risk of residual tumor after primary cytoreduction should be isolated in the preoperative period, and these should benefit from neoadjuvant chemotherapy. A glycoprotein, YKL-40, also known as chitinase-3-like 1 protein, was identified in the 1990s. Since then, it has been of special interest because it is associated with degradation of extracellular matrix and promotes angiogenesis in a vascular endothelial growth factor (VEGF)-independent manner[6,7]. During the last decade, YKL-40 has been increasingly studied in several tumor types[6,8-10]. *In vitro*, YKL-40 upregulates VEGF and is associated with tumor angiogenesis[9]. *In vivo* animal studies demonstrated that inhibition of YKL-40 decreased angiogenesis, tumor formation, and metastasis[11-13]. Recent studies showed that neutrophils and tumour cells expressed and released YKL-40 into the blood[9,14]. Increased serum levels of YKL-40 were shown in some cancer types such as breast cancer, lung cancer, colorectal cancer, melanoma, and endometrial cancer[6,7,10,15,16]. YKL-40 was suggested to have the potential to be a better marker than cancer antigen-125 (CA-125) for early diagnosis of EOC[17,18]. However, results from recent studies on the diagnostic efficiency of YKL-40 were inconsistent[18-20]. There are a limited number of studies reporting that elevated plasma YKL-40 levels are associated with worse outcomes in patients with ovarian cancer[17,21-23]. Also, it has been suggested that future studies should focus on determining an optimal cut-off value in patients with ovarian cancer for serum YKL-40[6]. In this study, we aimed to evaluate the usefulness of YKL-40 in the discrimination of benign and malignant adnexal masses and to determine the efficacy of YKL-40 in the preoperative estimation of the prognostic parameters such as stage and grade of the disease, residual tumor after primary cytoreduction, and response to platinum-based chemotherapy.

Materials and Methods

This prospective observational study was conducted at İstanbul University Cerrahpaşa Faculty of Medicine, Division of Gynecologic Oncology, between January 2015 and December 2017. The study was approved by the Ethics Committee of İstanbul University Cerrahpaşa Faculty of Medicine (protocol number: 83045809-604.01.02). Written informed consent was obtained from all patients. The manuscript was prepared in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology statement[24]. Blood samples were collected preoperatively from a nonconsecutive series of 100 patients who were planned to undergo surgery for an adnexal mass in our clinic. Exclusion criteria were the presence of one or more of the following: i) a suspicous malignancy other than ovarian cancer; ii) systematic disease including renal and/or hepatic impairment; iii) neoadjuvant chemotherapy; iv) history of any malignancy; v) ovarian malignancy other than serous histopathology; and vi) pregnancy. Frozen section evaluation was performed intraoperatively in the presence of suspicion in the diagnosis. The same gynecologic pathologists evaluated all of the specimens. The stage of disease and histologic types were in accordance with the International Federation of Gynecology and Obstetrics classification[25]. Maximal cytoreduction was defined as removing all gross tumoral tissue with no visible disease left. Optimal cytoreduction was defined as residual volume of 1 cm or less after surgery. Residual tumor more than 1 cm was classified as suboptimal cytoreduction. All patients with serous EOC (except those with stage IA and IB disease) received 6 cycles of adjuvant carboplatinum and paclitaxel. The platinum-free interval was defined as the interval from the last treatment with platinum to recurrence. Patients were accepted as platinum sensitive, if the platinum-free interval was longer than 6 months; platinum resistant, if it was shorter than 6 months; or platinum refractory, if the disease was persistent or progressive during the first-line chemotherapy. Progression of disease was diagnosed in the presence of elevated CA-125 and imaging results according to Response Evaluation Criteria in Solid Tumors criteria[26]. Progression-free survival was defined as the time interval between primary surgery and progression or death from any cause. Blood samples were collected in EDTA-containing tubes and anticoagulant-free tubes after an overnight fast on the morning before the surgery. Plasma and serum were seperated immediately and stored at -80 °C until analysis. After reaching the desired number of cases in both groups, all serum samples were defrosted at room temperature in the medical biochemistry laboratory of the faculty. Serum

YKL-40 concentrations were determined by a commercial enzyme-linked immunosorbent (ELISA) kit using a double-antibody sandwich enzyme immunoassay technique (human chitinase-3-like protein 1 YKL-40, ELISA Kit, Cat. No. YHB0684Hu; Shanghai Yehua Biological Technology Co. Ltd, China). Each ELISA analysis was performed according to the manufacturer's instructions. All tests showed intraassay and interassay coefficients of variations below 6% (n=10) and 7.5% (n=10), respectively. The analytical sensitivity of the test was 0.52 ng/mL.

Statistical Analysis

Patients' characteristics and clinical features were summarized using standard descriptive statistics. Mann-Whitney U test was used for comparison between two groups. T-test was used in comparison of independent samples' average. Receiver operating characteristics curves (ROC) were constructed for YKL-40 and CA-125 serum concentrations as diagnostics for cancer by plotting sensitivity versus 1-specificity, and area under curve (AUC) was calculated for both markers. All p-values were two sided and $p<0.05$ were considered as statistically significant. Statistical analyses were performed using SPSS version 21.

Results

Age, BMI, and menopausal status were similar between the malignant and benign groups (Table 1). The pathologic subtypes of benign ovarian tumors included endometriotic cysts (n=14), serous cystadenoma (n=12), dermoid cyst (n=9), and mucinous cystadenoma (n=5). Serum YKL-40 and CA-125 levels were significantly higher in patients with serous EOC, but CA-19-9 and CA-15-3 levels did not differ between the groups.

As illustrated in Figure 1, sensitivity was 80% and specificity was 70%, respectively, when a cut-off serum YKL-40 level of 47.7 ng/mL was applied. When 34.3 ng/mL was used as a cut-off value, the specificity decreased to 58.5%; however, sensitivity increased to 90%. The obtained areas under the ROC curves for YKL-40 (AUC=0.83) were greater than for CA-125 (AUC=0.75). Patients with serous EOC were analyzed seperately. One-quarter of the patients had stage I disease. Stage III disease consisted of almost 70% of the malignant group. In the next step, we analyzed the relationship between preoperative serum YKL-40 and clinicopathologic features of patients with serous EOC, such as stage, grade, residual tumor, and recurrence (Table 2). Significantly increased serum levels of YKL-40 were observed in patients with advanced stage and higher grade disease. When compared with maximal cytoreduction, optimal and suboptimal cytoreductions were found to be correlated with greater YKL-40 levels. The overall recurrence rate was 30% within a median follow-up time of 21 months (range, 2-35 months). The median time to recurrence after primary surgery and platinum-based chemotherapy was 7 months. The comparison of preoperative serum YKL-40 levels between patients with and without recurrence demonstrated signicificantly elevated levels in the latter patients (Table 2). When patients who had recurrence were investiagted in terms of platinum sensitivity, it was found that YKL-40 were significantly higher in the sensitive group compared with resistant and refractory groups (mean values of YKL-40: 179.2, 146.9 and 141.7 in platinum-sensitve, resistant, and refractory patients, respectively) (Figure 2).

Table 1. Clinical and laboratory characteristics of groups with malignant and benign disease

	Malignant group (n=60)	Benign group (n=40)	p
	Mean	**Mean**	
Age (years)	55.1	49.7	NS
BMI (kg/m^2)	26.6	27.1	NS
Menopausal status	**n (%)**	**n (%)**	
Premenopausal	19 (31.6)	14 (35)	NS
Postmenopausal	41 (68.3)	26 (65)	
	Mean	**Mean**	
YKL-40 (ng/mL)	102.9	43.9	<0.05
CA-125 (U/mL)	1137.5	151.9	<0.05
CA 19-9 (U/mL)	56.5	33.6	NS
CA 15-3 (U/mL)	72.5	18.5	NS

NS: Not significant, CA: Cancer antigen, BMI: Body mass index

Table 2. Clinical characteristics and plasma YKL-40 levels of patients with serous epithelial ovarian carcinoma

	n (%)	YKL-40 (ng/mL) Mean ± SD	p
Histology			
Serous	60 (100)	102.9±63.5	-
FIGO stage			0.001
IA-IC	15 (25)	50.6±23.6	
II	4 (6.6)	107.2±63.5	
IIIA-IIIC2	41 (68.3)	121.7±63.7	
Histologic grade			0.002
1	8 (13.3)	34.6±16.4	
2	2 (3.3)	156.6±133.7	
3	50 (83.3)	111.7±59.1	
Residual tumor			<0.001
0	51 (85)	94.1±57.4	
≤1 cm	7 (11.6)	136.8±38.3	
>1 cm	2 (3.3)	261.2±14.1	
Recurrence			<0.001
No	42 (70)	77.4±47.5	-
Yes	18 (30)	162.5±56.5	-

SD: Standard deviation, FIGO: International Federation of Gynecology and Obstetrics

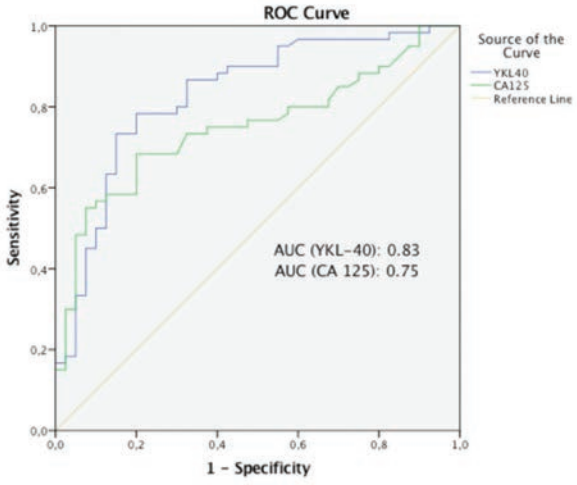

Figure 1. Receiver operating characteristic curves showing the performance of serum YKL-40 and cancer antigen-125 levels for differentiating between benign ovarian tumors and epithelial ovarian carcinoma

ROC: Receiver operating characteristic, CA-125: Cancer antigen-125, AUC: Area under curve

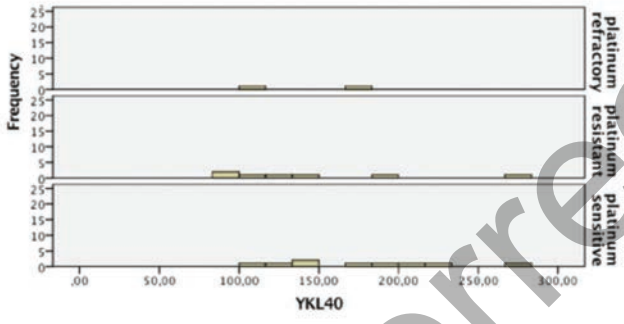

Figure 2. Receiver operating characteristic curves showing the performance of serum YKL-40 and cancer antigen-125 levels for differentiating between benign ovarian tumors and epithelial ovarian carcinoma

Discussion

There are still limited data investigating serum markers to differentiate between benign and malignant ovarian tumors[27]. For long time, the best documented and the most frequently recommended serum marker has been CA-125 in the management of adnexal masses[28]. In this study, preoperative serum levels of YKL-40 were assessed in patients with benign adnexal mass, and in patients with serous EOC. One of the major findings of this study is that patients with EOC had significantly higher serum YKL-40 levels compared with those with benign ovarian tumors. Moderate-to-high sensitivity and specificity (80% and 70%, respectively) was found when a cut-off level of 47.7 ng/mL was used. On the other hand, the sensitivity and specificity of CA-125 (cut-off value: 35 U/mL) were 76% and 57.5%, respecitvely. YKL-40 was a better predictor of ovarian cancer than CA-125 in our study population. Similar findings were observed in previous studies. Plasma YKL-40 levels were found to be elevated in more than 70% of patients with ovarian cancer compared with healthy patients[17,21-23]. As a result, YKL-40 has been included in the "top 9" biomarkers that are capable of discriminating between malignant and benign ovarian diseases[29]. Secondly, we investigated the association between YKL-40 and prognostic factors of EOC such as stage and grade of disease, residual tumor after primary cytoreduction, and recurrence. Results from similar studies suggested that high preoperative YKL-40 was an independent predictor of worse survival, which is in accordance with our findings[6,17,21-23]. Zou et al.[19] found that YKL-40 was associated with poor clinical outcome and worse tumor stages and grades. A unique study from Copenhagen, investigating the prognostic value of plasma YKL-40 in platinum-resistant ovarian cancer patients treated with bevacizumab found that elevated YKL-40 levels (>95th percentile) at baseline were associated with poor survial and residual disease after primary surgery[6]. Furthermore, two studies found that high serum levels of YKL-40 were associated with chemoresistance[22,30]. On the contrary, higher YKL-40 levels were detected in platinum-sensitive patients in the present study. One possible explanation for the contrast may be that Gronlund et al.[22] examined the role of YKL-40 on chemosensitivity in the second-line treatment of EOC patients, whereas we included only patients with serous EOC who received first-line platinum-based chemotherapy. For the first time, Chudecka-Glaz et al.[30] demonstrated that higher serum YKL-40 levels were associated with first-line platinum resistance. Their study and ours included a limited number of patients. Larger studies are needed to see if there is a correlation between YKL-40 and platinum sensitivity.

Study Limitations

It has been suggested that future studies of serum YKL-40 should be powered to investigate its value as a biomarker in individual histologic subtypes of ovarian cancer[6]. In our study, all patients with ovarian malignancy had the same histopathology. The present study included patients over a period of almost three years. However, because of the short study period, the same practices (same surgical team, same chemotherapy regimen starting on almost same postoperative days) were applied to all patients. The limitations of this study include the small number of patients, which means that strong conclusions cannot be drawn.

Conclusion

In conclusion, our findings suggest that preoperative serum YKL-40 can be used for to discriminate between malignant and benign ovarian tumors. In addition, higher YKL-40 levels indicate a higher risk of recurrence. Conflicting results regarding YKL-40 and platinum sensitivity should be eliminated by larger prospective studies.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul University Cerrahpaşa Faculty of Medicine (protocol number: 83045809-604.01.02).
Informed Consent: Consent form was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.K., N.T., V.Ş., H.T., M.A., F.D., T.B., Concept: İ.K., N.T., T.B., F.D., H.U., Design: İ.K., N.T., T.B., V.Ş., H.U., Data Collection or Processing: N.T., H.T., G.Ş., R.G., H.U., Analysis or Interpretation: H.U., İ.K., G.Ş., H.U., Literature Search: İ.K., V.Ş., H.T., H.U., Writing: İ.K., H.T.
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Women self-reported G-spot existence and relation with sexual function and genital perception

Kadınlara göre G-spot var mı? G-spotun seksüel fonksiyonlar ve genital algı ile ilişkisi

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Abstract

Objective: Aim of study to determine the existence of the G-spot from the healthy women's point of view and to assess the relationship with sexual function and genital perception.

Materials and Methods: Sexually-active healthy polyclinic patients aged between 18 and 54 years (n=309) were classified into three groups as group 1 (do not agree, n=90, 29.1%), group 2 (neutral/do not know, n=61, 19.7%) and group 3 (agree, n=158, 51.1%) with regard to participants' responses to a question of "does the G-spot exist." The Female Sexual Function index (FSFI) and Female Genital Self-Image scale (FGSIS) were administered to the participants.

Results: Half of the patients (51.1%, n=151) indicated that the G-spot exists. The groups were statistically homogeneous in terms of body mass index, parity, marital status, number of partners, and sexual orientation (p=0.41, p=0.06, p=0.12, p=0.19, p=0.25; respectively). Women with an education level of "less than high school" reported the absence of the G-spot significantly more often than others, whereas women with an education level of "university and higher" reported the presence of the G-spot more often (p≤0.001). Sexual dysfunction was found to be more frequent in group 1 when compared with group 3 (p=0.002, 67.8%, 45.6%). The orgasm subdomain scores of the FSFI and FGSIS total scores were significantly higher in group 3 than in group 1 (p<0.001, p=0.041).

Conclusion: Half of healthy women in the Turkish population believe that the G-spot exists. Those women showed better scores in sexual functioning and genital perception.

Keywords: Female Sexual Function index, Grafenberg's zone, G-spot, vulvar perception

Öz

Amaç: Bu çalışmanın amacı; sağlıklı kadınlara göre G-spot'un varlığını sorgulamak ve cevapların seksüel fonksiyonlar ve genital algı ile ilişkisini belirlemektir.

Gereç ve Yöntemler: Cinsel aktif 18-54 yaş aralığında sağlıklı poliklinik hastalarının; "G-spot var mı?" sorusuna verdikleri yanıtlarına göre grup 1 (katılmıyorum, n=90, %29,1), grup 2 (kararsızım/bilmiyorum, n=61, %19,7), grup 3 (katılıyorum, n=158, %51,1) şeklinde üç gruba ayrıldı (n=309). Hastalara FSFI (Kadın Cinsel İşlev ölçeği) ve FGSIS (Kadın Genital Algı ölçeği) uygulandı.

Bulgular: Hastaların yarısı G-Spot'un var olduğunu belirtti (%51,1, n=151). Vücut kitle indeksi, parite, evlilik durumu, partner sayısı, seksüel yönelim bakımından G-spot grupları arasında istatistiksel anlamlı fark saptanmadı (p=0,41, p=0,06, p=0,12, p=0,19, p=0,25). Lise ve daha az eğitimi olan katılımcılar daha yüksek oranda G-spot yoktur derken; üniversite ve üzerinde eğitim alan katılımcılarda, G-spot vardır diyenler anlamlı olarak fazlaydı (p≤0,001). Grup 3'teki kadınlarla kıyaslandığında, grup 1'de FSFI'ya göre cinsel işlev bozukluğu sıklığı daha yüksekti (%67,8, %45,6, p=0,002); FSFI orgazm alt grup skoru ve FGSIS toplam skorları grup 3'te grup 1'e kıyasla anlamlı olarak daha yüksekti (sırasıyla; p<0,001, p=0,041).

Sonuç: Türk popülasyonundaki sağlıklı yetişkin kadınların yarısı G-spot varlığına inanmaktadır. Bu kadınların cinsel işlev ve genital algı skorları daha fazla bulunmuştur.

Anahtar Kelimeler: Kadın Cinsel İşlev indeksi, Grafenberg bölgesi, G-noktası, vulvar algı

PRECIS: Women self-reported G-spot existence.

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Introduction

Female sexuality is complex and is influenced by many factors related to physiologic, psychological, hormonal, social, cultural, and partner issues. The vital organ in males is the penis, whereas the uterus, vagina, clitoris, and the Grafenberg-spot (G-spot), whose existence is not definite, are among the factors that are effective in women[1]. The G-spot is a current and controversial issue, and it now attracts interest in female sexuality because it involves a market share in genital esthetics with interventions such as its augmentation[2]. Ernst Grafenberg was the first to describe the G-spot as an erogenous zone approximately half a centimeter in size, below the urethra on the anterior wall of the vagina, but the first reports of its presence date back much further. During orgasm, this area is pressed downwards like a small cystocele protruding into the vaginal canal[3]. The G-spot was named after Addiego's case report on female ejaculation thirty years later[4]. In this article, it was stated that when a 1.5-2 cm area extending along the long axis of the urethra was touched, it gave rise to a urination feeling, and when stimulation was sustained, it was stimulated in sexual terms, and with this stimulation, the area was enlarged at a rate of 50%. About the same subject, anatomists, gynecologists, and sexual experts published self-reported questionnaire studies, case studies, anatomic and histologic studies and imaging studies[5-13]. Whether it is really anatomically present or a scientific deception still awaits an answer and publications are contradictory[14,15]. In this study, we asked in detail whether the participant felt a coin-size sensitive area in the anterior vaginal wall at the time of finger or penis penetration or pressure; namely the G-spot. The purpose of the study was to investigate how many women who as owners of this zone and to investigate its possible effect on sexual function and female genital perception.

Materials and Methods

The institutional Ethics Committee approved the study (Düzce University) (approval number: 2018/81), and written informed consent was obtained from all individual participants included in this study.

The descriptive cross-sectional study was conducted in a medical faculty between January 2018 and April 2018. The questionnaires were administered to healthy female participants who reported no known illnesses. Sexually-active and premenopausal patients ageg over 18 years who were admitted to our hospital polyclinic for routine gynecologic examinations were admitted to the study. Patients with esthetic concerns, those planning to undergo genital esthetic procedures, postmenopausal patients, those who had never had vaginal sexual intercourse, incontinence, pelvic organ prolapses, menstrual disorders and gynecologic cancer history, gynecologic surgery for any reason, oral contraceptive and antidepressant medication, with episiotomy, and those using intrauterine devices were excluded from the study. A total of 309 participants who agreed to participate in the study, who met these criteria, and who completed the questionnaire were included in the analyses of the study. Two lesbian participants were excluded from the study because they said they had not experienced any vaginal sexual intercourse, and five lesbian participants were included in the study because they stated that they engaged in vaginal sexual intercourse. The participants were taken into a quiet room, and their demographic data were recorded, the questions on the G-spot were asked, and the Female Sexual Function index (FSFI) and Female Genital Self-Image scale (FGSIS) questionnaires, which have been validated for the Turkish language, were administered[16,17]. The FSFI is a brief instrument for the assessment of sexual function consisting of 19 questions. It was validated based on the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (diagnoses of desire disorder, arousal disorder, and orgasmic dysfunction). Questions are scored for domains of libido, arousal, lubrication, orgasm, satisfaction, and pain[18]. Female sexual dysfunction was defined as a total score of 26 or less (maximum possible score of 36)[19]. To investigate the relationship between the G-spot and orgasm, the orgasm subdomain scores were also calculated (for orgasm subdomain, a maximum possible score of 6). Based on the fact that female sexual functions are associated with genital perception, we applied the FGSIS, which has been validated for the Turkish language[17]. The FGSIS is a 7-item questionnaire and is easy to apply, and shows female genital perception[20]. The question related to the G-spot asked was as follow: "Is there a region on the front of your vagina where you urinate and where you feel more sensitive when stimulated with a finger or penis during sexual intercourse?; Answers were collected in the form of "No, I do not agree," "I am undecided-I do not know," "Yes, I agree." According to the responses given to the G-spot questions, the participants were divided into groups 1, 2, 3; and then, the analyses were made.

Statistical Analysis

Continuous data are summarized as mean ± standard deviation and categorical data as frequency and percentage. The independent Samples t-test and One-Way analysis of variance were used to compare groups. Relations between categorical variables were examined using Pearson's chi-square or Fisher's exact tests. When significant results were found, subgroup analyses were performed with Bonferroni correction. Correlations between continuous data were analyzed using Pearson's correlation coefficient. The IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. (IBM, SPSS Inc., USA) statistical software package was used, and the significance level (p) was considered as <0.05.

Results

The demographic data of the patients are given in Table 1. Among all participants, there were 151 (51.1%) participants who said that the G-spot existed; 90 (29.1%) participants said

"No, there is no such region" and 61 (19.7%) participants said that they were indecisive or did not know. The mean age was 35.8±5.9 (minimum-maximum, 18-54) years. When the age groups were divided into categories as 18-24, 25-34, 35-44, 45-54 years, there were statistically significant differences between these age groups (p=0.03). In the subgroup analysis performed with Bonferroni correction, it was seen that the significant difference was only in the 45-54 age group, in group 2 and group 3. Regarding body mass index, parity, marital status, partner count, and sexual orientation, no statistically significant differences were detected between the G-spot groups (p=0.41, p=0.06, p=0.12, p=0.19, p=0.25). There were significant differences regarding levels of education (p≤0.001). It was observed that the university and more education group stated that the G-spot existed at a higher rate, and the group that consisted of high school and below levels of education stated that the G-spot did not exist at a higher rate. The FSFI and FGSIS comparative analyses with G-spot groups are shown in Table 2. FSFI total score averages were found as 21.0±8.9; 22.8±8.5; 24.8±8.6, respectively, according to the groups that did not agree with the existence of the G-spot, and those that were indecisive, and agreed. The total scores were statistically different between the groups (p=0.004). There was a significant difference (p=0.002) between the G-spot groups when the FSFI total score was divided into the two groups as those scoring below and above 26 (p=0.002). According to the post-hoc test result, this difference was found between group 1 and 3. It was also determined that those who were indecisive showed similarities in both groups. In terms of the FSFI orgasm subdomain, there were significant differences between the groups according to the G-spot agreement status (p<0.001); this difference was found between all groups according to the post-hoc test result. We also found that there was a significant difference between the groups in terms of FGSIS total scores (p=0.041). According to the post-hoc test result, this difference was between those who said that there was a G-spot and those who said that there was no such spot. It was also determined that those who were indecisive showed similarities between the groups. The FGSIS score was positively correlated at a weak level with both the FSFI (r=0.277, p<0.001) and the FSFI-orgasm (r=0.282; p<0.001).

Table 1. Self-reported G-spot existence among groups and demographic data

	G-spot does not exist (n=90)	I am not sure (n=61)	G-spot exists (n=158)	p
Age* (mean ± SD)	33.1±7.6	37.6±8.2	34.5±7.1	0.03*
18-24 y (n=31, 10%)	14 (15.6%)[a]	3 (4.9%)[a]	14 (8.9%)[a]	0.03**
25-34 y (n=121, 39.2%)	35 (38.9%)[a]	21(34.4%)[a]	65 (41.1%)[a]	
35-44 y (n=117, 37.9%)	34 (37.8%)[a]	22 (36.1%)[a]	61 (38.6%)[a]	
45-54 y (n=40, 12.9%)	7 (7.8%)[a]	15 (24.6%)[b]	18 (11.4%)[a]	
BMI (mean ± SD)	25.0±4.4	24.0±3.9	24.6±4.7	0.41
Parity (median, min-max)	2 (0-9)	1 (0-7)	1 (0-7)	0.06
Marital status (n, %)				
Single/partner (n=25, 8.1%)	10 (11.1%)	4 (6.6%)	11 (7%)	
Married (n=264, 85.4%)	75 (83.3%)	49 (80.3%)	140 (88.6%)	0.12
Divorced (n=20, 6.5%)	5 (5.6%)	8 (13.1%)	7 (4.4%)	
Partner count (n, %)				
One	88 (29.3%)	61 (20.3%)	151 (50.3%)	0.19
More than one	2 (22.2%)	0	7 (77.8%)	
Sexual orientation (n, %)				
Heterosexual (n=301, 97.4%)	87 (96.7%)	58 (95.1%)	156 (98.7%)	
Bisexual (n=3, 1%)	1 (1.1%)	2 (3.3%)	0	0.25
Lesbian (n=5, 1.6%)	2 (2.2%)	1 (1.6%)	2 (1.3%)	
Levels of education* (n, %)				
Less than high school (n=87, 28.2%)	41 (45.5%)[a]	16 (26.2%)[b]	30 (19%)[b]	
High school (n=46, 14.9%)	15 (16.7%)[a]	4 (6.6%)[a]	27 (17.1%)[a]	≤0.001**
University and higher (n=176, 56.9%)	34 (37.7%)[a]	41 (67.2%)[a]	111 (63.9%)[b]	

[a, b]Each subscript letter denotes a subset of categories whose column proportions do not differ significantly from each other.
([a]compared with [a,b]compared with b is not significantly different)

Table 2. Patients' self-reported G-spot existence and relation with Female Sexual Function index and Female Genital Self-Image scale

	G-spot does not exist (n=90)	I am not sure (n=61)	G-spot exists (n=158)	p
FSFI ≤26* n (%)	61 (67.8%)[a]	37 (60.7%)[a,b]	72 (45.6%)[b]	0.002
FSFI ≥26* n (%)	29 (32.2%)[a]	24 (39.3%)[a,b]	86 (54.4%)[b]	
FSFI total score (mean ± SD)	21.0±8.9	22.8±8.5	24.8±8.6	0.004
FSFI orgasm subdomain score (mean ± SD)	2.8±1.6	3.6±1.7	4.2±1.6	<0.001
FGSIS total score (mean ± SD)	20.7±3.8	20.9±4.1	21.9±3.9	0.041

[a,b]Each subscript letter denotes a subset of categories whose column proportions do not differ significantly from each other.
([a]compared with [a,b]compared with b is not significantly different. [a,b]compared with a or b is not significantly different with both)
FSFI: Female Sexual Function index, FGIS: Female Genital Self-Image scale

Discussion

Although the existence of the G-spot is usually accepted by the general public, it is anatomically controversial[5,7,14,21,22]. Biopsy studies showed that the anterior vaginal wall was more densely innervated than the posterior, and the distal region contained a higher number of nerve fibers than the proximal region[23]. Microdissection and immunohistochemical studies with seven fresh cadavers confirmed the abovementioned data and that the distal anterior vaginal wall was thicker than the proximal wall[24]. Although these data reveal the more sensitive and evident open-to-stimuli structure of the anterior vaginal wall, there are biopsy results showing the opposite viewpoint[5,7]. Ostrenzky first anatomically identified the neurovascular structure that he called the G-spot complex in a fresh cadaver in 2012[6]. Two years later, the same author published a cadaver study in which the histology of the G-spot complex was shown. In this study, the G-spot complex was detected anatomically in all eight cadavers, and the tissues were shown histologically by staining with hematoxylin and eosin in two randomized cases[22]. In a recent article by Hoag et al.,[7] which described the most extensive anatomic study of the anterior vaginal wall containing a detailed dissection of thirteen cadavers, the G-spot could not be described in the front wall of the vagina. In these two separate cadaver studies, it is confusing that the G-spot was defined at a rate of one hundred percent in one study yet the other study could not define it at a rate of one hundred percent. Studies are contradictory. In a study conducted by Puppo and Gruenwald[2], Puppo and Puppo[25] in which they reviewed the terminology of female sexuality, they wrote that the G-spot did not exist under the subtitle of "The G-spot does not exist: Is it a scientific fraud?" They stated that there was no vaginal orgasm and added that there was no scientific support for research that said the G-spot and vaginal orgasm existed. It was stated in some previous reserach that perhaps the G-spot was formed with a pudendal nerve innervation in areas that varied from person to person in the front side of the vagina instead of a same specific area in everybody[1]. In a previous study that was conducted by asking questions to patients, there were 1234 participants in the first large-scale G-spot self-reported questionnaire study. In this study, the G-spot identification rates were determined as high as 84.3%. It was determined that approximately 3 years after the age of the first relationship, people were found to have reached orgasm through sexual intercourse, and they discovered the sensitive region of the vagina about 6 years after the first orgasm[11]. One year later, the self-reported G-spot study of the same group, which included 1289 patients, revealed a G-spot detection rate of 82%. In this study, however, the sampling consisted of nurses, sex therapists, and counselors who had a high-level of education and who were very familiar with these issues[12]. The high rates may be due to the nature of the sampling. In another self-reported questionnaire that was recently published, the rate of expressing G-spot existence was 56%. In this study conducted on twins to investigate the genetic basis of G-spot existence, no genetic basis was found. The reason for this may be that people cannot discover their G-spot via environmental factors[10]. In this study, the oldest patient was aged 83 years, and the rate of elderly participants who stated that the G-spot existed was lower. This result is not surprising. In our study, postmenopausal participants were excluded from the study and it was seen that the value that was found to be significant was in the 45-54 years' age group, and in the group that was indecisive, which we considered having no clinical significance. In our study, the rate of participants who believed in the existence of the G-spot was found as 51.1%. This ratio is compatible with the literature[10]. The percentage of those who thought that there was a G-spot in the university graduate group was high. In a similar study, there was no difference between the levels of education and the responses[10]. In our study, the difference between the levels of education and the G-spot groups could be due to the change in the understandability of the problem with education. Another possibility is that college graduates who use the right resources for accessing information more accurately can have higher G-spot awareness or greater exposure to media. People may have discovered their bodies better by reading and practicing what they read. Filling materials such as hyaluronic

acid, autologous oil injections, and G-spot augmentations are rapidly increasing worldwide today. In a case report with autologous fat injection, no change was determined in the sexual function questionnaire before and after the application and there was no increase in experiencing orgasms[26]. The vagina is a dynamic organ that plays an active role in sexual intercourse. Anatomic relationships and dynamic interactions between the clitoris, urethra, and anterior vaginal wall have led to the concept of a clitourethrovaginal complex, which defines a versatile, functional area that may induce orgasmic responses when properly stimulated during penetration[27]. It is emphasized that this means a broader meaning beyond a spot. In another study, a strong and reverse relation was found between the distance of a woman's clitoris and her urethral meatus. It was emphasized that this result was secondary to more stimuli due to the increased pressure on the vaginal wall and nerve extensions of the clitoris into the vagina[28]. On the same subject, another study under the title of "echography of the G-Spot" measured the urethrovaginal space thickness using introital ultrasonography, and the association with vaginal orgasm was examined; it was found that this measurement was directly related to vaginal orgasm[29]. Those who think that G-spot exists have higher genital perception and sexual function scores compared with other participants. In light of the above studies, it is not wrong to claim that women are more vaginally stimulated when they feel that there is a G-spot. For this reason, it is not surprising that these women's sexual functions, especially orgasm subdomains, are high. In this group, another reason that the genital perception scores may have been perceived as high might be due to the fact that the sexual functions were good in this group, higher than the group that claimed that there was no G-spot. In our study, there was a positive correlation between FGSIS and FSFI, as it was in the original study of the genital perception questionnaire. It is known that the self-image of the person affects sexual functions, which proves this[20]. The FGSIS has a positive correlation with all subdomains except the desire domain of FSFI[30]. In our study, the weak positive correlation could be attributed to the multifactorial nature of female sexuality.

A person's exploration of sexuality is a process, and the fact that they do not know the sensitive areas of the vagina may mean that such areas do not exist in reality as well as that one has not yet discovered these areas. This can prolong this process in countries where sexuality is a taboo subject of discussion, where experiences before marriage are few and the possibility of having sexual experience with different partners before marriage is low. Partner incompatibility is another factor in this subject. For this reason, the fact that the participants do not know the existence of the G-spot or are indecisive about its existence does not mean that this point does not actually exist in reality. The fact that the present study was self-reported and the sample being small are limitations. The possibility of not understanding the question is another limitation. In further investigations, in addition to the self-reported questionnaires in wider series, the aim is to determine the location of the G-spot with a finger during an examination and compare the self-reported answers with the examination findings.

G-spot presence continues to be an interesting subject in the academic environment and for the public. The biggest reason for this might be that there is no consensus on its existence. This issue will continue to attract interest until definitive and descriptive studies are made.

Conclusion

Half of the participants stated that G-spot existed, which was consistent with the literature. An increase in sexual function, orgasm scores, and genital perception scores of these women was identified. Self-reported questionnaires give an idea of G-spot existence but are inadequate as proof. Further histologic and anatomic studies are needed with larger series.

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Ethics

Ethic Approval Number: The institutional Ethics Committee approved the study Düzce University (approval number: 2018/81), and written informed consent was obtained from all individual participants included in this study.
Informed Consent: Written informed consent.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: A.E.K., E.Ç., Design: A.E.K., E.Ç., Data Collection or Processing: A.E.K., E.Ç., Analysis or Interpretation: A.E.K., E.Ç., Literature Search: A.E.K., E.Ç., Writing: A.E.K., E.Ç.
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Active management of the third stage of labor: A brief overview of key issues

Doğumun üçüncü evresinin aktif yönetimi: Kilit konulara kısa bir bakış

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Abstract

Postpartum hemorrhage is a potentially life-threatening, albeit preventable, condition that persists as a leading cause of maternal death. It occurs mostly during the third stage of labor, and active management of the third stage of labor (AMTSL) can prevent its occurrence. AMTSL is a recommended series of steps, including the provision of uterotonic drugs immediately upon fetal delivery, controlled cord traction, and massage of the uterine fundus, as developed by the World Health Organization. Here, we present current opinion and protocols for AMTSL.

Keywords: Postpartum hemorrhage, active management of the third stage of labor, uterotonic agents

Öz

Postpartum kanama, hayatı tehdit eden, önlenebilir bir durumdur, anne ölümünün önde gelen nedenini oluşturan bir durumdur. Çoğunlukla doğumun üçüncü evresi sırasında ortaya çıkar ve doğumun üçüncü evresinin aktif yönetimi (AMTSL) ortaya çıkmasını engelleyebilir. AMTSL, uterotonik ilaçların hemen fetal doğum üzerine uygulanması, kontrollü kordon traksiyonu ve Dünya Sağlık Örgütü tarafından geliştirilen rahim fundus masajı dahil olmak üzere önerilen bir dizi adımdır. Burada AMTSL için güncel görüş ve protokolleri sunuyoruz.

Anahtar Kelimeler: Doğum sonrası kanama, doğumun üçüncü evresinin aktif yönetimi, uterotonik ajanlar

Introduction and Definitions

Postpartum hemorrhage (PPH) and blood loss complications constitute one of the most common causes of maternal mortality and morbidity. PPH is defined differently in various countries (Table 1)[1-4]. Its incidence is 11% globally among women in labor[1-4]. The third stage of labor (TSL) is defined as the time between the delivery of the baby and the expulsion of the placenta. The duration of the third stage is ~6-30 minute[3,4]. The pathophysiology of the TSL is still not fully understood. During this stage, expulsion of placenta with the formation of capillary hemorrhage after the birth of the baby is followed by shrinking of the placental surface with uterine contractions, and finally ends with the discharge of the placenta from the uterus. Hemorrhage is restricted with uterine contractions and activation of the coagulation system[2-4]. As can be understood from this definition, some degree of hemorrhage always occurs at this stage (~100-250 cc). It is important to limit the amount of hemorrhage to the minimum possible level. Accordingly, the World Health Organization (WHO) suggested the active management of the TSL (Table 2)[3].

Uterotonic agents

1) Oxytocin: Oxytocin is the most commonly used agent and the primary drug of choice in the TSL. Oxytocin's action is unique to the smooth muscles of the uterus; it increases the amplitude and frequency of contractions. Oxytocin binds to a G-protein on the surface of uterine myocytes, resulting in the generation of diacylglycerol (DAG) and

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Table 1. Post-partum hemorrhage definitions

Country	Definition
Australia 2008	Amount of blood loss: >500 mL at vaginal birth, >750 mL at cesarean section
Austria 2008	Amount of blood loss between 500-1000 mL + clinical symptoms of hypovolemic shock or amount of blood loss >1000 mL
Germany 2008	Amount of blood loss during first 24 h after birth ≥500 mL, serious PPH blood loss during first 24 h after birth ≥1000 mL
Royal College of Obstetrics and Gynecology, United Kingdom	Amount of blood loss of 500-1000 mL and absence of clinical symptoms of hypovolemic shock Amount of blood loss ≥1000 mL or presence of clinical symptoms of hypovolemic shock
World Health Organization	Amount of blood loss during first 24 h after birth ≥500 mL, serious PPH blood loss during first 24 h after birth ≥1000 mL

Table 2. Active management of the third stage of labor

a) Administration of uterotonic agents after delivery of the baby
b) Expulsion of placenta with controlled traction of cord
c) Uterine fundal massage after expulsion of placenta

inositol triphosphate (IP3) via the action of phospholipase C on phosphatidylinositol bisphosphate. DAG stimulates PG synthesis, and IP3 stimulates the release of calcium from the sarcoplasmic reticulum. It also activates cyclooxygenase 2 by a further G-protein interaction and, in doing so, stimulates PG synthesis.

Oxytocin can be used just after delivery of the front shoulder of the baby or expulsion of the placenta. Generally, its administration route and dose are 10 IU intramuscularly (IM). It can also be used intravenously (IV), which is typically preferred during cesarean sections (CS). A newly developed oxytocin tablet has recently been presented that can be applied successfully via the sublingual route[5]. An *in vitro* study showed a >30% reduction in tissue transepithelial electrical resistance after treatment with the oxytocin fast-dissolving tablet, implying an increase in the permeability of the mucosal tissue to oxytocin[5]. However, it may cause ST depression on an electrocardiogram and hypotension. The efficacy of oxytocin is the same with both administration routes. In a 600-patient study from Turkey, there was no statistically significant difference in the amount of postpartum blood loss between IM and IV administration[6]. Oxytocin also decreased postpartum blood loss when applied inside the placental cord[7].

2) Ergometrine (methergine): Ergot alkaloids exert various effects throughout the body on at least three different types of receptor. They are non-selective 5-hydroxytryptamine 1 agonists and have affinities for dopamine and noradrenalin receptors. Ergot alkaloids are absorbed rapidly and completely after oral administration. Both are usually effective within 1-5 min after an IM injection. They are metabolized in the liver, and reported half-lives range from 0.5 to 2 h. Their actions on the uterus are probably a result of their agonist properties against adrenergic α-receptors; these receptors, when stimulated, lead to IP3 release and to calcium mobilization from the sarcoplasmic reticulum. To date, there is only one prospective study in the English literature on this topic. In that study, the authors compared the efficacy of rectal misoprostol 400 µg, oxytocin 10 IU injected IM, methylergometrine 0.2 mg injected IV, and 0.5 mg ergometrine +5 IU oxytocin injected IM in reducing blood loss in the TSL. They found that methylergometrine had the "best" uterotonic drug profile (lowest blood loss during the third stage and duration of the TSL). However, the study had several limitations. Most importantly, it was not a randomized study, the trial was not double-blinded, leading to the possibility of biased results, and no power calculation was reported[8]. Ergometrine causes continuous contraction of the uterus. There is not enough evidence about its use as a single agent. It is typically administered at 0.2 mg IM. Its use must be avoided in patients with hypertension.

3) Syntometrine: This contains 5 IU oxytocin and 0.5 mg ergometrine. The time of onset of the uterine response after IM administration is shorter than after ergometrine alone, and the duration of action is several hours. Although it was found to be more effective than oxytocin in a review, the adverse effect profile (hypertension, nausea, vomiting) restricts its use[9].

4) Misoprostol: This is a synthetic prostaglandin E1 derivative. It is an inexpensive drug and is stored readily. It does not cause high blood pressure and can be used in patients with asthma. Its most common adverse effect is flushing. Although the amount of blood loss has been shown to have been reduced with prophylactic use of misoprostol in many studies, it is not as effective as oxytocin. Consequently, oxytocin is the first choice for the prophylaxis of PPH[10-12]. In countries where the socioeconomic level is very low and home deliveries are common, misoprostol can be used as the first-line drug; it can be used orally, rectally or sublingually. The route of administration and dose differ from country to country. The WHO and International Federation of Gynaecology and Obstetrics recommend a single dose of 600 µg misoprostol,

oral or sublingual, for the prophylaxis of PPH[13]. According to a recently published meta-analysis result, misoprostol has been used in the third stage of labor to prevent PPH when a sterile syringe and trained midwife were absent [14]. In a prospective randomized trial published in 2016, it was shown that the additional use of buccal misoprostol in conjunction with active management of the TSL reduced the need for additional uterotonic drugs[15].

5) Tranexamic acid: Tranexamic acid (TA) is a lysine derivative with anti-fibrinolytic activity that inhibits fibrin degradation by blocking lysine-binding regions on plasminogen. TA is absorbed from the gastrointestinal tract at 30-40%[16,17]. Its plasma half-life is 2 h, and its plasma protein binding ratio is ~3%, which is solely a result of plasminogen binding. It can cross the placenta and be passed to a breastfeeding infant. It is excreted through the urine, so it must be avoided in patients with renal failure. It can be used in an oral, local or parenteral manner. It is typically a well-tolerated drug. Rarely, it may cause nausea, vomiting, hypotension, and dizziness[17,18]. These adverse effects are more common when it is given parenterally at high flow rates. One gram of IV TA given within 3 hours of PPH was reported to significantly reduce maternal death and the need for surgery[19]. In pill form, it is recommended at a 15-25 mg/kg/dosage every 8 h orally for 5-10 days. There may be gastrointestinal adverse effects. The maximum dose is 3-4 g. It should be given at 10 mg/kg/dosage (maximum 500 mg) with slow infusion every 8 h when given parenterally. Activated prothrombin complex concentrates must be avoided[17,18]. Using TA for the treatment of PPH, the incremental cost-effectiveness ratios were found below the lower bound of the cost-effectiveness threshold range[20].

- Tranexamic acid for the prevention of postpartum hemorrhage after cesarean section

There are 11 randomized controlled trials (RCT) about this topic in the literature[21-31]. Except in one study, elective CSs were performed in all patients. In all studies, the amount of blood loss decreased with the use of TA and no adverse effects were reported. The first large study was reported by Gungorduk et al. [24] from Bakırköy Women and Children's Hospital in 2011. In total, 660 patients were included in the study and a decrease in the amount of blood loss was seen after the routine administration of 5 IU oxytocin following 20 IU oxytocin in 500 ml RL and TA (1 g IV in 5 min) at the third stage of labor; no adverse effects were reported[24]. Another study with 740 patients reported similar results in 2013[29]. As can be seen from the results of these studies, administration of TA as an additional agent in the TSL decreased the blood loss.

- Tranexamic acid for the prevention of prevention of postpartum hemorrhage after vaginal delivery

There are three RCTs on this topic in the literature[24,32]. Yang et al. [32] administered 10 IU oxytocin 10 min after delivery of TA in their study. They reported that TA decreased the incidence of PPH. In a study published in 2013 from Kanuni Sultan Süleyman Training and Research Hospital, İstanbul, Turkey, the TSL was managed actively in all patients. An additional 1 g TA was given to a group of patients in this study. At the end of the study, less blood loss and a lower incidence of PPH was reported in the group using TA[24]. Mirghafourvand et al. [33] reported that administration of 1 g TA after 10 IU oxytocin decreased PPH in a study of 120 pregnant women in 2015. In conclusion, TA decreases the amount of blood loss and the incidence of PPH in patients who are managed actively in the TSL.

Tranexamic acid adverse effects

In a previous study, the risks of myocardial infarction, cerebrovascular stroke, deep vein thrombosis, and pulmonary embolus were not statistically significantly increased compared with a control group[16]. The most common adverse effects are nausea and vomiting. Two patients experienced thromboemboli when receiving high-dose TA in the EXADELI study[34]. There is only one reported RCT concerning neonatal adverse effects in the postpartum period. No adverse effects related with TA was reported in this study[24]. However, the design of the study was inadequate to assess neonatal adverse effects. It can cross the placenta, so there is a need for large studies with administration before cord clamping.

Route of administration for uterotonic agents

Although the standard doses of uterotonic agents are given above, the actual dose and administration forms differ in various countries. Table 3 lists four common international guidelines for dose and administration[29,35].

Table 3. Drug doses and administration forms in four major international guidelines

	ACOG	RANZCOG	RCOG	SCOG
Oxytocin	10-40 IU IV or 10 IU IM	Not specified	5 IU IV (repeatable) or 40 IU IV 125 mL/h in 500 mL	10 IU IM or 5 IU IV or 40 IU IV 125 mL/h in 500 mL
Ergots	Metilergonavin 0.2 mg IM per 24 h period	Not specified	Ergometrin 0.5 mg IV or IM	Ergonovin 0.25 mg IV or IM every 2 h
Misoprostol	800-1000 µg rectal	1000 µg rectal	1000 µg rectal	400-1000 µg oral or rectal
Tranexamic acid	-	-	Not suggested	Not suggested

ACOG: American Congress of Obstetrics and Gynecology, RANZCOG: Royal Australian and New Zealand Congress of Obstetrics and Gynecology, RCOG: Royal Congress of Obstetrics and Gynecology, SCOG: Canadian Society of Obstetrics and Gynecology, IV: Intravenous, IM: Intramuscular

Delivery of placenta with controlled cord traction

Although this was recommended in the 2007 WHO guidelines, it is described as optional for the active management of the third stage in the 2012 updated guidelines[13]. An inexperienced operator may cause serious complications, such as uterine inversion. In WHO studies, it was accepted as ineffective for decreasing blood loss. However, according to a meta-analysis reported in 2015, although the risk of blood loss above 1000 mL was not decreased with controlled cord traction, the mean time of the third stage, the mean blood loss (less than 10 mL), and the risk of blood loss less than 500 mL were all decreased[36]. The authors noted that controlled cord traction still had a place in active management when performed by experienced personnel. It is also a recommended method for CSs[24,37].

Uterine fundal massage after placental expulsion

Uterine fundal massage after placental expulsion provides uterine contractions by stimulating endogenous prostaglandin secretion. This method was recommended in the 2007 WHO guidelines, and was described as optional for the active management of the third stage in the 2012 updated guidelines[13]. Similarly, Chen et al. [38] published results of a study of 2340 pregnancies, which showed that the addition of fundal massage to oxytocin did not decrease PPH.

Possible adverse effects of active management

Adverse effects related to uterotonic agents
- Hypertension, nausea, vomiting due to ergot alkaloids

Risk of placental retention

Neonatal risks related to early cord clamping
- Iron-deficiency anemia
- Intraventricular hemorrhage
- Hypotension

Results

According to the most recent Cochrane analysis, active management of the TSL decreases the risk of postpartum bleeding of over 1000 mL[39]. The possible risks and benefits of active management must be explained to pregnant women and informed consent must be obtained. TA administration, as an extra drug for pregnant women whose TSL is being managed actively, decreases both the amount of blood loss and the incidence of PPH.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.O., **Design:** Y.O., **Literature Search:** V.G., M.K., **Writing:** K.G.

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

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Review / Derleme

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Use of a gelatin-thrombin hemostatic matrix in obstetrics and gynecologicl surgery

Obstetrik ve jinekolojik cerrahide jelatin-trombin hemostatik matriks kullanımı

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Abstract

Gelatin-thrombin matrix (GTM) is a hemostatic sealant consisting of bovine-derived gelatin matrix and human-derived thrombin, combining both mechanical and active mechanisms to achieve hemostasis. It was approved by the Food and Drug Administration in 1999. GTM has been used by several surgical specialties; however, it is a possibly an under-used tool in obstetrics and gynecology. A limited number of studies have been performed on its use during laparoscopic endometrioma excision and myomectomy. It may prove useful in endometrioma excision in reproductive aged women because it is likely to harm ovarian reserve less than electrocautery; however, this conclusion needs to be validated. The only study on GTM use in myomectomy included 50 women randomized into GTM and control groups, and showed decreased blood loss and shorter hospital stays in the GTM group. In gynecologic oncology, it was successfully used to reduce lymphocele cases in a cohort study. GTM has been used successfully in obstetrics in a handful of cases of uncontrolled bleeding from caesarean scar, placental site, ectopic pregnancy, rectovaginal hematoma, and venous plexus over the vaginal vault after emergency postpartum hysterectomy. Risk of viral transmission is a major concern about GTM, yet there are no reports on disease transmission with GTM use to date. Rare but serious adverse effects and complications have been reported such as fatal or near-fatal thromboembolism and small bowel obstruction. Although GTM is mostly a safe product, it is still not free of complications and risks. In conclusion, although routine use of GTM cannot be recommended due to concerns about its safety, cost, and availability, it may prove useful when conventional hemostatic methods such as suturing and electrocauterization fail or are not appropriate.

Keywords: Hemostatic matrix, hemostasis, gelatin-thrombin hemostatic matrix, obstetrics, gynecological surgery

Öz

Jelatin-trombin matriks (GTM), hemostaz elde etmek için hem mekanik hem de aktif mekanizmaları kullanan, sığır kaynaklı jelatin matriks ve insandan türetilmiş trombin içeren hemostatik bir yalıtkandır. 1999 yılında Gıda ve İlaç Dairesi tarafından onaylanmıştır. GTM birçok cerrahi uzmanlık alanları tarafından kullanılmıştır, ancak obstetrik ve jinekolojide muhtemelen GTM'den yeterince faydalanılmamaktadır. Laparoskopik endometrioma eksizyonu ve miyomektomi sırasında kullanımı konusunda sınırlı sayıda çalışma yapılmıştır. Üreme çağındaki kadınlarda over rezervine elektrokoterden daha az zararlı olma olasılığı nedeniyle endometrioma eksizyonunda yararlı olabilir ancak bu sonucun doğrulanması gerekir. Miyomektomide GTM kullanımı üzerine yapılan tek çalışmada, 50 kadın GTM ve kontrol grupları olarak randomize edilmiş ve GTM grubunda kan kaybının azaldığı ve hastanede kalış süresini kısaldığı gösterilmiştir. Jinekolojik onkolojide, bir kohort çalışmasında lenfosel olgularını azalttığı görülmüştür. GTM, sezaryen skarından kaynaklanan kontrol edilemeyen kanamalar, plasental yatak kanamaları, ektopik gebelik, rektovajinal hematom ve vajinal venöz pleksuslardan kanama olan obstetrik olgularda başarılı bir şekilde kullanılmıştır. Viral bulaşma riski, GTM ile ilgili büyük bir sorun teşkil etmektedir, ancak bugüne kadar GTM kullanımı ile hastalık bulaşı konusunda herhangi bir olgu bildirilmemiştir. Ölümcül ya da morbiditesi yüksek tromboembolizm ve ince bağırsak tıkanıklığı gibi nadir fakat ciddi yan etkiler ve komplikasyonlar bildirilmiştir. GTM çoğunlukla güvenli bir ürün olmasına rağmen komplikasyon ve risklerden arınmış değildir. Sonuç olarak, GTM'nin rutin kullanımı, güvenlik, maliyet ve kullanılabilirliği ile ilgili endişelerden dolayı önerilemese de, dikiş ve elektrokoterizasyon gibi geleneksel hemostatik yöntemler başarısız olduğunda ya da uygun olmadığında yararlı olabilir.

Anahtar Kelimeler: Hemostatik matriks, hemostaz, jelatin-trombin matriks, obstetrik, jinekolojik cerrahi

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Introduction

Intraoperative hemorrhage remains a major concern of surgery in obstetrics and gynecology. Morbidity can be severe, resulting in increased transfusion rates, hospital stay, cost and rarely mortality. Although traditional methods of maintaining hemostasis (i.e., compression, suture ligation, electrocautery) usually suffice, they are not always successful or safe. In these situations, there is a need for alternative methods for achieving hemostasis. Hemostatic sealants (HS) have been developed to fulfill this need, and today a wide array of products is available. According to their mechanisms of action, they can be classified into mechanical sealants, flowable sealants, fibrin/synthetic sealants, and sealants with active ingredients[1]. There are more than 20 commercial products on the market, and more currently in development. A commonly used combination of bovine-derived gelatin matrix and human-derived thrombin [FloSeal Hemostatic Matrix (FloSeal) Baxter Healthcare Corporation Fremont, CA 94555, USA], has both mechanical and active ingredients to achieve hemostasis[2,3]. We will use the abbreviation gelatin-thrombin matrix (GTM) for, GTM in the rest of the text. GTM has been successfully used in several surgical specialties such as urology,[4,5] neurosurgery,[6-9] cardiovascular surgery,[10,11] orthopedic surgery,[12,13] and otorhinolaryngology;[14-17] however, its use in obstetrics and gynecology is not similarly well-documented. This review aims to focus on the properties and use of GTM in obstetrics and gynecology.

Properties and mechanism of action

The gelatin matrix is created by gelatinization of collagen extracted from bovine corium. The collagen fibers are cross-linked and stabilized with glutaraldehyde, and the thrombin is extracted from pooled human plasma. These two components are packaged separately, stored at room temperature, and mixed just prior to use[2,18]. GTM has two mechanisms of action (Figure 1 and Figure 2). First, the gelatin matrix fills the bleeding site with gelatin granules and swells, generating a stable clot. The gel conforms to the contour of the wound, through asymmetrical or irregular surfaces, providing a tamponade effect. A spontaneously forming clot also triggers contact activation of platelets, contributing further to hemostasis. Next, the extrinsic thrombin component of GTM converts fibrinogen into a fibrin polymer, which promotes fibrin formation at the end of the coagulation cascade. Over the course of 6-8 weeks, the GTM granules are absorbed without any residue[18,19].

It is important to note that GTM functions only in the presence of fibrinogen in the clot. Therefore, it is effective

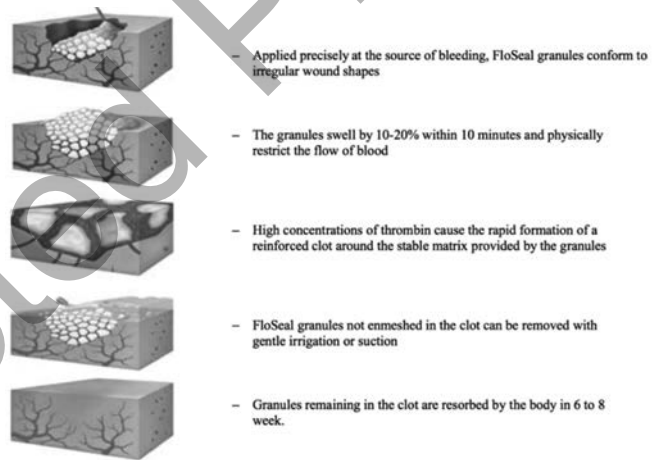

Figure 2. Gelatin-thrombin hemostatic matrixTM mechanism of action (Courtesy of Oz et al.[19] used with permission)

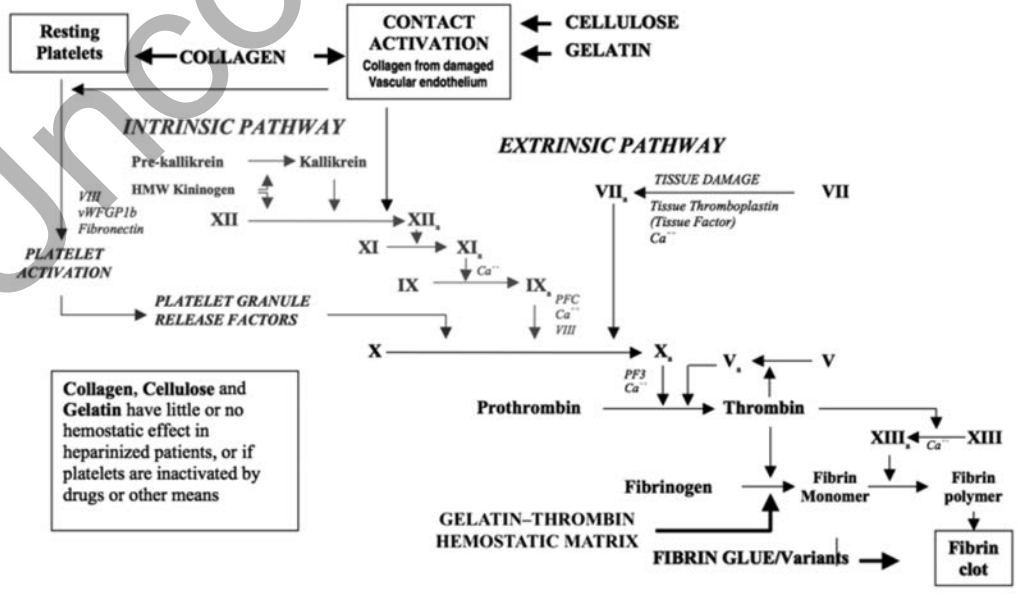

Figure 1. Coagulation cascade and hemostatic technologies (Courtesy of Oz et al.[18] used with permission)

in active bleeding sites exclusively. Excess product should be removed to avoid swelling. Nevertheless, care should be taken because aggressive irrigation, suction or any action that could disrupt or remove the clot itself because it would nullify the effect of GTM[2,18,19]. Finally, its application or injection into blood vessels should be strictly avoided because GTM intravasation may result in thromboembolism, as discussed in the "safety and adverse effects of GTM in gynecological surgery" section[20]. GTM was approved by the United States Food and Drug Administration (FDA) in 1999[21].

Gelatin-thrombin matrix in gynecology and obstetrics

GTM is a possibly an under-used tool in obstetrics and gynecology, as reflected in the paucity of literature regarding the related use of this substance. Studies are only available for a handful of indications, and a substantial number of publications are case reports.

Ovarian cystectomy

Most of the studies on GTM in gynecologic surgery are in the context of ovarian cystectomies. Unlike the other investigations on GTM, their primary outcomes are the prevention of blood loss and the preservation of the ovarian reserve. Angioli et al.[22] were the first to investigate the effectiveness of GTM during laparoscopic endometrioma excision. In their pilot study, they used GTM for hemostasis in the first 8 patients and bipolar forceps or carbon-dioxide laser in the following 12 patients with symptomatic endometriomas measuring ≥3 cm. Hemostasis was achieved in all patients within 3 minutes with a median time of 172 and 182 seconds in the control and GTM groups, respectively (p=0.19). Although the average blood loss was less in the GTM group, the difference was short of being statistically significant (p=0.37). Growing evidence shows a detrimental effect of endometrioma excision on ovarian reserve[23-28]. Alternative methods are being investigated because a possible factor is the use of electrocautery for hemostasis[29]. After being proven effective in endometriomas by Angioli et al.[22] GTM was compared with electrocautery in three randomized controlled trials (RCT). Sönmezer et al.[30] recruited 30 women with a unilateral endometriomas ≥4 cm. Despite being allocated to GTM, two patients required bipolar cautery for hemostasis and were excluded from the analysis of ovarian reserve. Preoperative and postoperative hemoglobin levels were comparable between the groups. Anti-müllerian hormone (AMH) levels were measured preoperatively, and at the first and third post-operative months. One month after surgery, the decrease in AMH was significantly higher in the bipolar cautery than the GTM group (56% vs. 29%, respectively, p=0.001); however, it was not significantly different at the third month (23% vs. 19%, respectively, p=0.467). In another RCT including 100 patients, Song et al.[31] compared AMH levels between GTM and electrocautery at the third post-operative month following laparoscopic excision of endometriomas. Three patients in the GTM group required bipolar coagulation and two patients in the bipolar cautery group needed ovarian suturing for hemostasis. Estimated blood loss was similar in both groups (67.3± 49.9 mL in the bipolar cautery group vs. 55.9±45.4 mL in the GTM group, p=0.22). However, the percentage decline in AMH levels was significantly higher in the bipolar cautery group than in the GTM group (16.1% vs. 41.2%, respectively; p=0.004). The third RCT included 60 women with bilateral endometriomas[32]. Either GTM or bipolar coagulation was used for hemostasis, and serum AMH levels were compared between the groups at the third post-operative month. Women in the GTM group had a significantly higher mean AMH level than the bipolar cautery group (1.68±0.32 ng/dL vs. 1.08 ±0.32 ng/mL). It should be noted that this trial remained as an abstract and did not develop into a full-text article; therefore, detailed information about the methods and results are not available. In our systematic review and meta-analysis of the effect of hemostatic methods on ovarian reserve following laparoscopic endometrioma excision, we found that although bipolar cautery caused a significantly greater decline in AMH compared with alternative methods, namely GTM and suturing [95% confidence interval (CI)=-13.00, -0.90], the difference was not significant in the subgroup analysis comparing bipolar coagulation and GTM (95% CI=-14.07, 2.53)[29]. We concluded that although the latter was possibly a false-negative finding due to a small sample size and there was moderate quality evidence supporting its use, we were still hesitant to suggest the widespread application of GTM due to its marginal benefit, additional cost, and possible adverse effects. To the best of our knowledge, there are no published studies about the use of GTM during the excision of other types of ovarian cysts, except for a case report by Ebert et al.[33] about stripping an ovarian serous cystadenoma.

Myomectomy

Even though myomectomy is a commonly performed procedure that can cause significant blood loss, there is limited data on the use of GTM in myomectomy. Raga et al.[34] randomized 50 women with symptomatic fibroids larger than the size of a 16 weeks' pregnant uterus who were undergoing conventional myomectomy into GTM and control groups. GTM or sterile saline was applied to the fibroid bed immediately after the removal of the fibroid and before uterine wound closure. Blood loss was estimated as the sum of the weight change in the gauzes and the blood volume in the suction bottle. The average intraoperative blood loss was 80±25.5 mL (range, 25-150 mL) and 625±120.5 mL (range, 250-950 mL) for the GTM and control groups, respectively (p<0.005). Likewise, average postoperative blood loss, measured by surgical drains, was significantly more in the control group (25±5 mL vs. 250±75 mL, p<0.005). Although none of the women in the GTM group required blood

transfusion, 5 patients were transfused in the control group. All of this translated into longer hospital stays for the control group ($p<0.005$). The small sample size and possible lack of blinding are drawbacks of this study and it is unfortunate that no other trial has been performed to support or oppose its findings.

Gynecologic oncology

It is interesting that the use of GTM has not been reported much in gynecologic oncological surgery, a field where hemostasis is of utmost importance and bleeding from or around many vital organs or tissues is expected. Yet, its use in pelvic lymph drainage and wound healing has drawn some attention.

Han et al.[35] reported the case of an 86-year-old woman who had undergone wide radical excision and bilateral inguinal lymphadenectomy for vulvar cancer. She had bilateral inguinal wound separation and excessive lymphorrhea postoperatively. Application of GTM resulted in successful granulation formation. Perfect wound healing and no drainage from the groin was reported two months later.[35]

In addition to this case-report, there is only one cohort article that studied the effect of GTM for the treatment pelvic lymphoceles. In this study, 50 patients underwent pelvic +/- paraaortic lymphadenectomy for various gynecologic cancers. In the study group, 5 mL GTM and its spray form (Coseal®) was used at the lymphadenectomy side instead of a pelvic drainage system. Pelvic drainage systems were used in the control group. Application of GTM decreased the hospital stay and the number of symptomatic lymphoceles in patients with gynecologic malignancies[36].

Ectopic pregnancy

Another scenario where a gynecologic surgeon can encounter uncontrolled bleeding is ectopic pregnancy. Although most tubal pregnancies are managed successfully with salpingectomy or salpingostomy, additional measures may be required to control bleeding. Clapp and Huang[37] reported two cases of tubal ectopic pregnancies where electrocautery failed to achieve hemostasis and GTM was used successfully. Interestingly, Watrowski[38] reported two cases of tubal pregnancies managed through salpingostomy where they used only GTM for hemostasis. Due to its cost, limited availability, and yet-to-be-proven efficacy, this approach is far from being the standard. It remains a viable option in patients who wish to preserve their fallopian tubes. Gorry et al.[39] and Watrowski et al.[40] reported cases of primary peritoneal pregnancy and primary omental pregnancy where GTM was used successfully to control bleeding.

Obstetrics

The literature on the use of GTM in obstetrics is limited to a few cases in the context of postpartum hemorrhage. Moatti et al.[41] described a case of massive post-partum hemorrhage forming a rectovaginal hematoma that could not be controlled with conventional methods; application of GTM with packing provided hemostasis. Another case, uncontrolled bleeding from the venous plexus along the vaginal vault after emergency postpartum hysterectomy in a patient with disseminated intravascular coagulopathy was managed successfully with GTM[42]. A patient with acute fatty liver of pregnancy who presented with acute hepatic and renal failure along with a hypofibrinogenemia was noted to have bleeding after vaginal delivery by vacuum extraction. This was controlled with intrauterine, vaginal application of GTM and recombinant activated human factor VIIa transfusion[43]. Finally, GTM proved successful in a woman with post-partum hemorrhage due to vaginal laceration that could not be controlled using traditional techniques due to "poor tissue quality"[44]. Similar cases of bleeding from a caesarean scar[45] and placental site[46,47] were controlled with GTM. These reports show that GTM can be an option in post-partum hemorrhage in cases where traditional methods fail.

Safety and adverse effects of gelatin-thrombin matrix in gynecologic surgery

As with any other product that contains human or animal derived components, GTM poses a theoretical risk of viral transmission. Although this risk can be reduced by screening donors and tracing cattle, it cannot be ruled out with current technology[6]. There are no reports of disease transmission from the currently available GTM products in the literature. Thromboembolism is another major concern about GTM and HS in general. Fatal pulmonary thromboembolism has been reported following the use of GTM during spinal surgery in a 78-year-old woman[20]. She developed dyspnea with right-sided heart failure due to left pulmonary artery embolization 8 hours after surgery. Autopsy revealed that the thrombus in the pulmonary artery contained acellular eosinophilic granules with enclosed fibrin and thrombocytes, convincing the pathologists that thromboembolism was a result of embolization of GTM granules from the application site to the pulmonary artery. The authors suggested that continuous uptake of small amounts of GTM through small vessels around the paravertebral site was the cause of thromboembolism. The injury to the vessel wall might have triggered the coagulation cascade that caused the migration of GTM granules. Therefore, surgeons should be careful about the risk of intravascular thrombus formation when GTM is used around relatively large-sized vessels.

Another case report involving an 18-year-old woman who developed peri-operative disseminated intravascular coagulation and acute right-sided heart failure that occurred during spinal surgery immediately after the application of an absorbable gelatin powder mixed with bovine thrombin. This event was attributed to unintentional intravasation of HS[48]. Small bowel obstruction (SBO) is another potential serious complication after application of GTM or similar HS in the

peritoneal cavity[49-52]. SBO following its use in gynecological surgery was first reported in 2009[51]. GTM was applied after iatrogenic injury to the inferior vena cava during laparoscopic lymphadenectomy for endometrial cancer. The patient developed nausea, vomiting, and abdominal pain on the 6th postoperative day and bowel sounds were absent. She was initially managed conservatively, but diagnostic laparoscopy was required on the 11th postoperative day. Adhesions were seen on the GTM application site only and this was thought to be the obvious cause of obstruction. A 15-cm small bowel segment was resected. Pathologic evaluation showed significant fibrotic changes caused by a foreign material, in accordance with a GTM product. Suzuki et al.[52] reported two cases of laparoscopic gynecologic procedures complicated by SBO, possibly related to the use of a hemostatic agent. In the first case, a 44-year-old woman who underwent laparoscopic myomectomy, a single dose of GTM (4 mL in total) was administered to the hysterotomy site for persistent oozing. She was re-admitted with severe pelvic pain on 4th postoperative day. In the second case, total laparoscopic hysterectomy and bilateral adnexectomy was performed and GTM was applied to control the bleeding from the left pelvic side wall after adhesiolysis. In both cases, SBO was noted at the GTM application sites during diagnostic laparoscopy (Figure 3). The authors concluded that triggering of an allergic reaction and formation of eosinophilic granulomatous tissue may result in intraperitoneal adhesions and SBO[52]. In the light of these case reports, it is suggested to wait for two minutes after GTM application and remove the excess material with gentle irrigation to decrease the risk for developing granulomatous tissue, which could result in SBO. These reports show that although GTM is mostly a safe product, it is still not free of risk. These are important considerations before its use because safer alternatives such as suturing or compression are available. GTM is a hemostat consisting of bovine-derived gelatin matrix and human-derived thrombin, combining both mechanical and active ingredient mechanisms to achieve hemostasis. Although it cannot be recommended as the first-line method due to concerns about safety, cost, and availability, it may be useful when conventional hemostatic methods such as suturing and electrocautery fail or are not appropriate. Moreover, it may prove useful in endometrioma excision in reproductive aged women because it is likely to harm ovarian reserve less than electrocautery. However, this should be validated with high quality studies. AThis research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

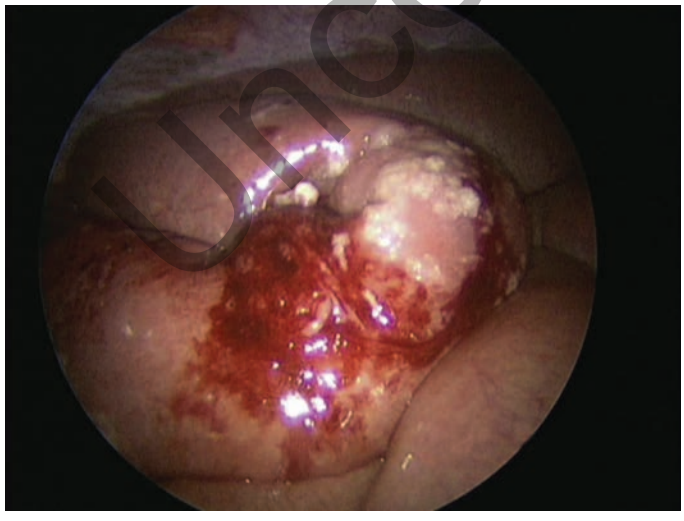

Figure 3. Granulation tissue and significant inflammatory reaction seen at the site of bowel adhesions (Courtesy of Suzuki et al.[52] used with permission)

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Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.M., E.T., Concept: B.U., B.A., Design: B.U., B.A., Data Collection or Processing: S.M., E.T., Analysis or Interpretation: S.M., E.T., B.A., Literature Search: H.Y., Writing: S.M., E.T., B.A.,

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Case Report / Olgu Sunumu

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Are complicated monochorionic twins more susceptible to indomethacin-induced fetal ductal constriction? Two cases of laser surgery for Twin-Twin Transfusion syndrome

Komplike monokoryonik gebelikler indometazinin neden olduğu fetal duktus arteriozus konstriksiyonuna daha mı duyarlıdır? İkizden İkize Transfüzyon sendromu nedeniyle lazer cerrahisi yapılan iki olgu

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Abstract

Indomethacin is a commonly used medication against preterm delivery. Several reports of fetal ductal constriction have been described after indomethacin use in the literature; however, there are no previously documented reports describing an association between Twin-Twin Transfusion syndrome and a constrictor effect of indomethacin on the ductus arteriosus. Two patients were referred to our department for Twin-Twin Transfusion syndrome and each underwent placental laser surgery. Constriction of the ductus arteriosus occurred as early as 20 and 24 weeks' gestation following maternal use of indomethacin after laser surgery. Spontaneous amelioration was observed after discontinuation of the drug. The constrictor effect of indomethacin on the ductus arteriosus can be observed even after a single dose and as early as 20 weeks of gestation in complicated monochorionic twin pregnancies. We emphasize meticulous use of indomethacin in complicated monochorionic twin pregnancies because the constrictive effect seems to be independent of gestational age.

Keywords: Constriction, ductus arteriosus, indomethacin, preterm labor, Twin-Twin Transfusion syndrome

Öz

İndometazin preterm eylem durumunda sıklıkla kullanılan bir ilaçtır. Literatürde indometazin ve duktus arteriozus konstriksiyonu ile ilgili olgular rapor edilmişse de ikizden ikize transfüzyon sendromu ile ilişkisini vurgulayan bir çalışma yoktur. Hastanemize İkizden İkize Transfüzyon sendromu tanısı ile iki olgu refere edilmiştir ve bu olgulara plasental lazer cerrahisi uygulanmıştır. Cerrahi sonrası uterin kontraksiyonları engellemek amacıyla indometazin tedavisi verilmiştir ve 20 ve 24. gebelik haftasında olan bu olgularda duktus konstriksiyonu gelişmiştir. Tedavi kesildikten sonra klinik tablo kendiliğinden düzelmiştir. Komplike olan monokoryonik ikiz gebeliklerde indometazinin duktus arteriozus üzerindeki konstriktor etkisi tek doz sonrası bile 20. gebelik haftası gibi erken bir gebelik haftasında gözlenebilir. İndometazinin duktus üzerine olan etkisi gebelik haftasından bağımsız olduğundan komplike monokoryonik ikiz gebeliklerde indometazin dikkatli kullanılmalıdır.

Anahtar Kelimeler: Konstriksiyon, duktus arteriozus, indometazin, preterm doğum, İkizden İkize Tansfüzyon sendromu

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Introduction

Monochorionic (MC) twins are a subtype of monozygotic (MZ) pregnancies and are seen in 0.3% of all pregnancies. MC diamniotic pregnancies are the most common subtype of MZ pregnancies and account for 70-75% of all MZ pregnancies[1]. Twin-Twin Transfusion syndrome (TTTS) is a complication of MC twin pregnancies and foetoscopic laser photocoagulation is one of the treatment options; however, laser surgery increases the risk of abortion/preterm delivery. Indomethacin is a commonly used medication against preterm delivery. Several reports of fetal ductal constriction have been described after indomethacin use in the literature. Here, we present two cases of antenatally diagnosed foetal ductal constriction in the donor co-twin after indomethacin use.

Case Reports

Case 1

A 24-year-old gravida-3-para-2 at 24 weeks' gestation was referred to our department for TTTS. Her physical and gynecologic examinations were usual. Fetal examination revealed a diamniotic MC twin pregnancy. The recipient twin was diagnosed as having polyhydramnios and the donor twin had oligohydramnios. The diagnosis of stage 3 TTTS was made on the basis of Quintero Staging system. A negative ductus venosus A-wave was observed in the recipient twin. The deepest vertical pocket was 140 mm for the recipient and 5 mm for the donor twin. The complications and prognosis of TTTS and risks of placental laser surgery were discussed with the family and the patient opted for placental laser surgery. The patient underwent placental laser surgery under local anaesthesia in operating room. On the day of the operation, 100 mg indomethacin was administered due to regular uterine contractions as rectal suppository once a day. Indomethacin administration was continued until postoperative day 2 and the contractions disappeared. On postoperative day 3, an ultrasound examination revealed pleural effusion, ascites, increased nuchal thickness, oligohydramnios, tricuspid regurgitation, and negative ductus venosus A-wave in the donor twin and normal Doppler findings in the recipient twin. A detailed examination of the ductus arteriosus showed ductal narrowing with a transverse diameter of 1.49 mm (<5th percentile) (Figure 1)[2]. There was no turbulent flow or aliasing in the ductus arteriosus region and systolic and diastolic velocities were 69 and 6 cm/s, respectively. Although Doppler criteria of ductal constriction were not observed, hydrops was prominent. The indomethacin treatment was stopped. The direction of ductus venosus flow turned to positive on the next day and further examination revealed a 50% increase in the transverse diameter of the ductus arteriosus, and the ductal arch returned to its normal shape (Figure 2). The tricuspid regurgitation and ascites, and pleural effusion disappeared completely on postoperative

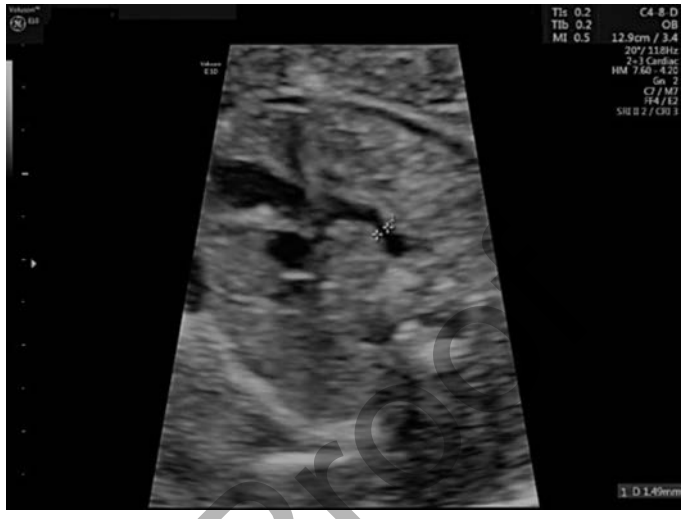

Figure 1. Narrowing in the ductus arteriosus (Case 1)

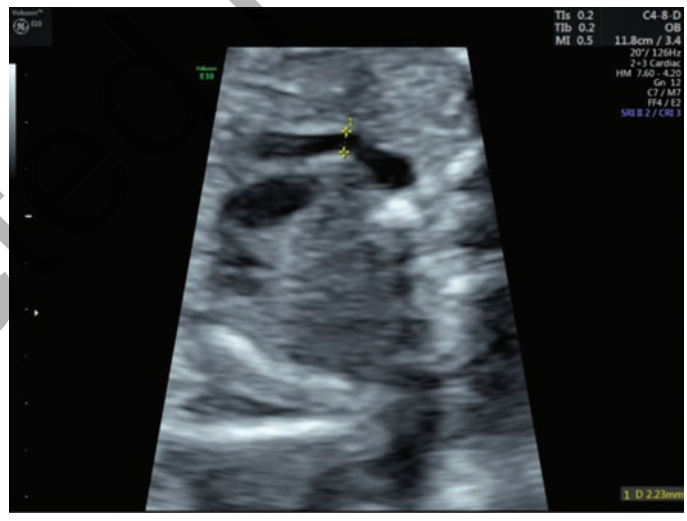

Figure 2. Transverse diameter of ductus arteriosus after resolution of the constriction (Case 1)

days 5 and day 7, respectively. The patient was discharged on tenth day postoperatively. Her antenatal visits were uneventful until delivery. The patient underwent a cesarean section for breech-vertex presentation at 35 weeks' gestation. Male infants weighing 2200 and 2100 g were delivered.

Case 2

A 25-year-old gravida-2-para-1 at 20 weeks' gestation was referred to our department for TTTs. The patient reported abdominal bloating. The diagnosis of TTTs stage 2 was made on the basis of Quintero staging. The deepest vertical pocket was 154 mm for the recipient and 10 mm for the donor twin. The estimated foetal weight (EFW) was less than 10th centile for donor twin. Placental laser surgery was performed after preoperative counselling. Postoperatively, a single-dose 100 mg indomethacin suppository was inserted into the rectum to prevent uterine contractions. On the next day (postoperative

day 1), constriction of the ductus arteriosus and tricuspid regurgitation were observed in the donor twin. There was a marked ductal narrowing (Figure 3). The peak systolic velocity was 149 cm/s (Figure 4). On postoperative day 3, the transverse diameter of the ductus arteriosus and peak systolic velocity returned to normal (Figure 5). On postoperative day 5, the tricuspid regurgitation had disappeared and the patient was discharged. On the next day, the patient was admitted to the hospital due to regular contractions and cervical opening and she delivered at 21 weeks and 3 days' gestation.

Discussion

Preterm delivery is a major cause of perinatal morbidity and mortality. Indomethacin is relatively old and the most commonly used prostaglandin synthetase inhibitor, it has been used in preterm delivery since the 1970s. Maternal use of indomethacin is the most common cause of foetal ductus arteriosus constriction; however, idiopathic constriction of the fetal ductus arteriosus has also been described in the literature[3]. The diagnostic criteria of ductal constriction are as follows: a) presence of turbulent flow with a continuous pattern in the ductus arteriosus region (systolic and diastolic) in colour Doppler; b) systolic velocity ≥1.4 m/s; and c) diastolic velocity ≥0.3 m/s[4]. To the best of our knowledge, this is the first report of prenatal diagnosis of constriction of ductus arteriosus in as early as 20 and 24 weeks' gestation following maternal use of indomethacin in complicated MC twins after laser surgery. Prostaglandin inhibitors such as indomethacin are tocolytic agents, which act by decreasing prostaglandin activity in the uterine myometrium. However, decreased prostaglandin synthesis in the foetal circulation may have adverse effects on the ductus arteriosus because prostaglandins mainstay ductal patency. Fetal ductal constriction is a distinct entity caused by different drugs (non-steroidal anti-inflammatory drugs, fluoxetine or abuse of some drugs); however, idiopathic/spontaneous constriction of foetal ductus arteriosus has also been described in the literature[5]. Tricuspid regurgitation, right ventricular dilatation, oligohydramnios, ascites, hydrops, and increased nuchal translucency are common foetal findings in such cases. A case series reported 45 cases of fetal ductus arteriosus constriction and closure, including 8 idiopathic forms. The most common finding of ductal constriction was tricuspid regurgitation, present in 86.6% of patients[5]. Although indomethacin was administered as a single dose at 20 weeks' gestation and once a day for 2 days at 24 week's gestation, our cases presented with constriction of the ductus arteriosus. As we know from previous reports, constriction of the ductus arteriosus is not dependent on serum indomethacin levels[6]. Vermillion et al.[7] analysed the effect of indomethacin tocolysis on fetal ductus arteriosus constriction of 72 fetuses and reported that the greatest incidence of ductal constriction occurred at 31 weeks' gestation. The earliest gestational age for ductal constriction was reported at 24.7

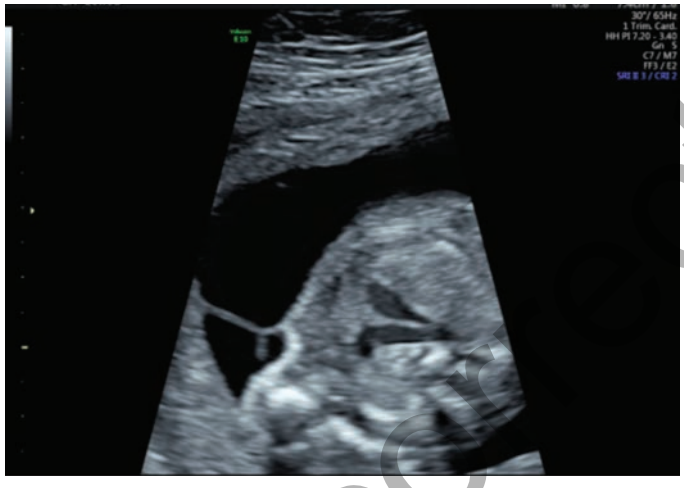

Figure 3. Narrowing in the ductus arteriosus (Case 2)

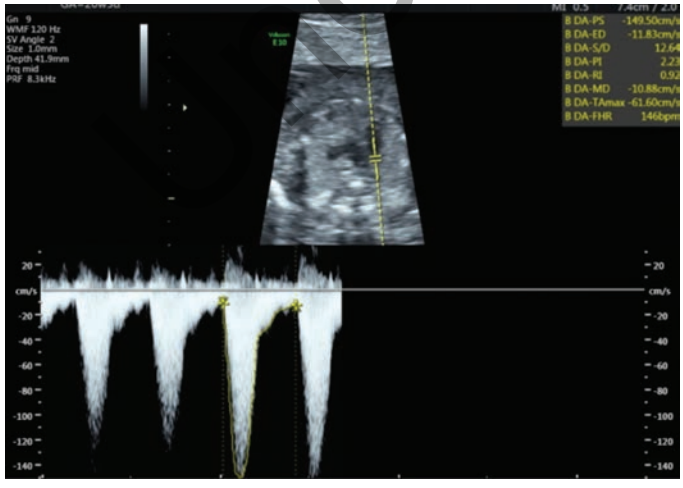

Figure 4. Elevated peak systolic velocity in the ductus arteriosus (Case 2)

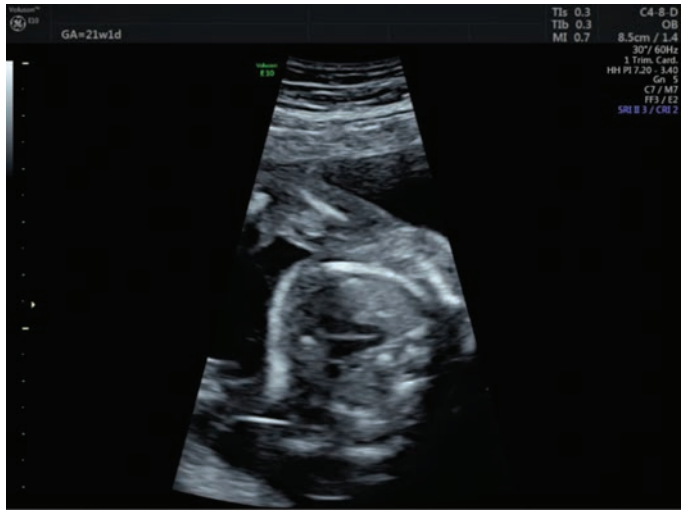

Figure 5. Transverse diameter of the ductus arteriosus after resolution of the constriction (Case 2)

weeks' gestation[7]. Therefore, it could be argued that the ductal effect of indomethacin is unpredictable for the foetus at any gestational age. Complete and irreversible closure of the ductus arteriosus can be observed even after a single dose of indomethacin, whereas ductus arteriosus can remain unaffected despite repeated doses. In our cases, constriction of the ductus arteriosus was seen only in the donor twin. In our opinion, this effect was observed only in the donor twins because of the relatively hypoxic conditions of donor foetuses. A selective constrictive effect of indomethacin on MC twins has been shown previously in some pathologically proven reports[8]. Furthermore, indomethacin can decrease fetal urine output and lead to deterioration in amniotic fluid levels in the donor foetus. Thus, it seems reasonable to use tocolytics other than indomethacin in TTTS. Just a few cases of hydrops and constriction of ductus arteriosus following indomethacin use have been described in the literature and none presented as early as 24 weeks' gestation. As in our case, hydrops resolved after discontinuation of indomethacin[9]. Indomethacin use is associated with a risk of constriction of the ductus arteriosus at any gestational age; however, this condition and its effects can present in the absence of Doppler criteria. In case 1, the absence of Doppler criteria can be explained by the relatively early gestational age and severe tricuspid regurgitation. Tricuspid regurgitation allows the right ventricle to empty and may prevent turbulent flow in the ductus arteriosus. In conclusion, these are the first reported cases of foetal ductal constriction in complicated MC twin pregnancy presenting as early as 20 weeks' of gestation. Therefore, we emphasize meticulous use of indomethacin in complicated MC twin pregnancies because the constrictive effect seems to be independent of gestational age. In the event of indomethacin use, Doppler indices of the ductus arteriosus should be closely monitored.

Ethics

Informed Consent: Was taken.
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Authorship Contributions

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Case Report / Olgu Sunumu

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Primitive neuroectodermal tumor of genital tract in hysterectomized patient: A case report

Histerektomize bir kadında genital primitif nöroektodermal tümör: Olgu sunumu ve literatür derlemesi

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Abstract

Primitive neuroectodermal tumors are high-grade malignant neoplasms. These are uncommon entities for the female genital tract. The treatment, management and follow-up period of Ewing's tumors are not well-defined because of their rarity in the genital tract. Surgical debulking is the mainstay treatment in all cases. After debulking surgery, patients receive chemotherapy and/or radiotherapy and there is a relation between disease stage and survival. Herein, we present a case of ovarian primitive neuroectodermal tumor with a review of previously reported cases.

Keywords: Ovary tumor, primitive neuroectodermal tumor, Ewing's tumor

Öz

Primitif nöroektodermal tümörler yüksek dereceli malign kanserlerdir. Kadın genital sisteminde nadir görülür. Ewing tümörleri, genital sistemde nadir görüldüğü için, tedavi, yönetim ve takip periyodu iyi tanımlanmamıştır. Debulking cerrahi bütün olguların tedavisinde temeldir, cerrahi sonrası hastalar kemoterapi ve/veya radyoterapi alabilirler. Hastalığın derecesi ve sağkalım arasında ilişki vardır. Bu yazıda, ovaryan primitif nöroektodermal tümör olgusu sunup, literatürdeki diğer olguları da kapsayan bir derleme hazırladık.

Anahtar Kelimeler: Yumurtalık tümörü, primitif nöroektodermal tümör, Ewing tümörü

Introduction

Primitive neuroectodermal tumors (PNETs), which are known as Ewing's sarcoma (ES), are high-grade malignant neoplasms that develop from a group of neuroectodermal small round cells[1]. The typical locations of PNETs are around the skeletal system, but they can arise from any soft tissue[2]. ES is an uncommon condition in the female genital tract;[3] ovarian tumors with primitive neuroectodermal components for postmenopausal women are extremely rare and only a few cases have been reported. Herein, we present a case of ovarian PNET with review of previously reported cases.

Case Report

A 64-year-old woman, gravida 2, para 2, presented with pelvic pain, which she had had for approximately four months. She underwent a ventro-suspension 25 years ago for uterine prolapse. However, a re-operation for uterine prolapse consisting of laparoscopy-assisted vaginal hysterectomy was performed 3 years ago. During this procedure, the uterus was separated from bilateral cornual regions and adnexae were left. The result of a pathologic evaluation was reported as benign for the uterus corpus material but wide cervical intraepithelial grade 3 neoplasia signs for the cervix were reported. A physical examination revealed a pelvic mass fixed to the left anterolateral abdominal wall. Abdominal magnetic resonance imaging revealed a huge mass in the pelvic cavity backward the bladder with irregular borders. The tumor markers were carbohydrate antigen (CA)-125; 269.7 kU/L (reference value; 0-35 kU/L). She underwent a debulking operation with bilateral salpingoopherectomy and total omentectomy, bilateral pelvic and paraaortic lymph node dissection, appendectomy, and aspiration for cytologic evaluation. The left ovarian mass had invaded

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the abdominal wall and resection of the fascia and part of the rectus abdominis muscle was needed; a polypropylene mesh was used to close the abdominal wall. There was no visible tumor after surgery. The tumor was characterized by a proliferation of small, round, primitive cells with a diffuse growth pattern. The cells had scant cytoplasm, irregularly-shaped and hyper-chromatic nuclei with coarse chromatin and a brisk mitotic rate. In some areas there were perivascular pseudorosette-like structures. The histology showed round cells with hyper chromatic nuclei and pleomorphisms, eosinophilic cytoplasm, very frequent mitosis, apoptosis, and focal necrosis. The tumor showed diffuse, strong, cytoplasmic and membranous CD56, nuclear Fli-1 positivity. Multifocal staining for neuron specific enolase (NSE) and mesothelin and focal high molecular weight (HMW)+low molecular weight cytokeratin (CK), epithelial membrane antigen (EMA), synaptophysin (SYNP), WT1 positivity was detected (Figure 1, 2). The tumor cells were also positive for p53. CD99, chromogranin A, CD45, inhibin, calretinin, CA-125, ER, PR, CK7, CK20, Moc31, Tag72, myogenin, S100, and destine were negative. The surgical specimens of one ovary, appendix, and omentum were interpreted as Ewing sarcoma/PNET after immunohistologic and histologic studies. The patient was referred to the medical oncology department and chemotherapy consisting of vincristine, cyclophosphamide, and cisplatin was started. Radiotherapy was not applied. The CA-125 value was 84.4 U/mL before her first chemotherapy. The patient completed six chemotherapies after surgery. There was no evidence of disease after 7 months of follow-up.

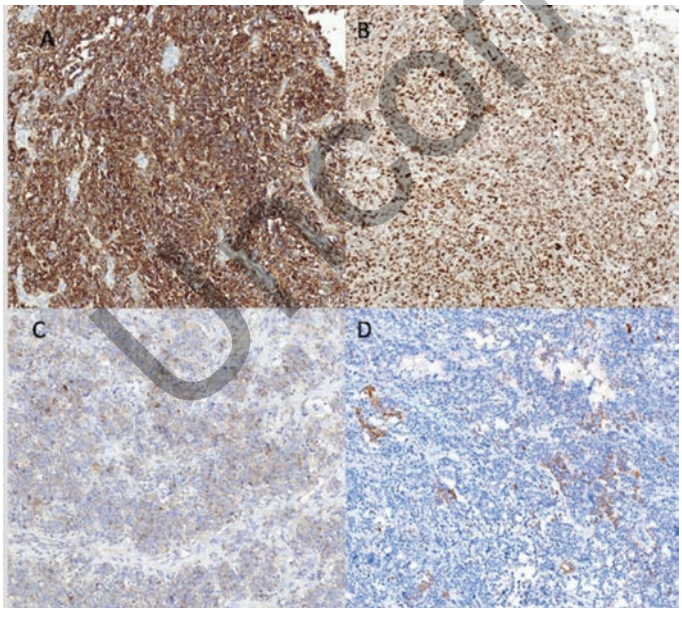

Figure 1. A) Strong membranous and cytoplasmic CD56 positivity (CD56*10), B) Nuclear Fli1 positivity (Fli1*10), C) Focal cytoplasmic SYNP staining (SYNP*10), D) Focal HMW+LMW CK positivity*10

Discussion

PNETs are uncommon entities especially for the female genital tract and the ovaries are the most common location[1-15]. It seems that the exact age for non-skeletal ES is not clear, but cases in the literature were seen between the second and third decades of the life. The first postmenopausal patient with ovarian PNET was reported by Fischer et al.[16]. According to our knowledge, this case is the second ovarian Ewing's tumor to be diagnosed in the postmenopausal period. For our patient, invasion to the anterolateral abdominal wall may have been due to suspension to this region of the round ligament and adnexa during the operation for uterine prolapse many years ago. To date, nearly 30 ovarian ES cases have been reported and some of these are summarized in Table 1.[6-11,17-22] PNETs have poor prognosis and due to the fact that tumors have an aggressive potential, survival periods are short. Two reported cases in literature had six years' survival. Age at diagnosis is meaningful for five-year overall survival ranges. As reported in the case of Fischer et al.[16] the patient was alive for six months. Therefore, our patient is one of the rare cases to be diagnosed in postmenopausal period and has the longest survival period. The treatment, management, and follow-up periods for Ewing's tumors in the genital tract are not well-defined because of their rarity. Until recently, 18 cases of uterus corpus, 5 cases of cervix uteri, 3 cases of vulvar, and 4 cases of vaginal location have been reported[23]. Uterine abnormal bleeding and enlargement of the uterus size were the main symptoms reported for uterine corpus and cervix uteri ES-PNETs, and painless, nodular vulvar masses were typical for the vulvar or vaginal tumors. Among these cases, tumor markers had increased values in nearly all patients. Surgical debulking was the mainstay of treatment in all cases. After debulking

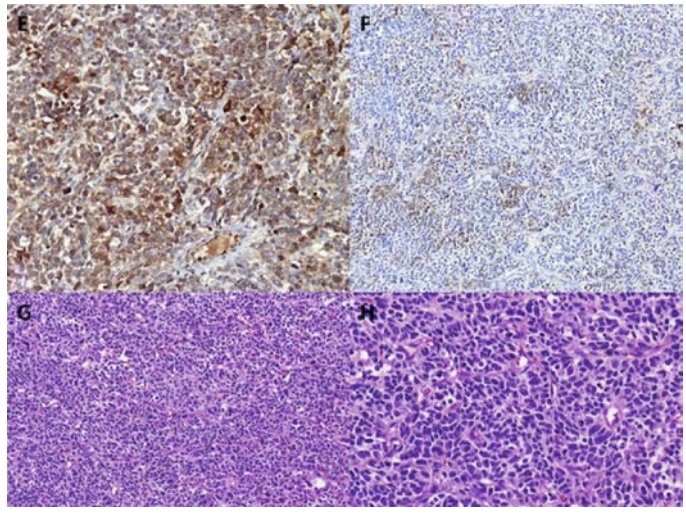

Figure 2. E) Neuron spesific enolase staining (*10), F) Epithelial membrane antigen staining (*10), G, H) Hematoxyline-eosin staining (*10)

Table 1. Clinicopathological features of primary ovarian Ewing's sarcoma-primitive neuroectodermal tumor

Reference	Pathology	Ag e	Stage	Treatment	Follow up	Recurrence
Winkler et al.[6]	Central PNET	37	Advanced	Supracervical hysterectomy+BSO+omentectomy+lymph node diss+suboptimal debulking+chemotherapy	2 months	Acid, bulky lymph nodes, peritoneal disease
Yousefi et al.[7]	PNET	43	Advanced	Debulking surgery	Pulmoner metastase died	
Lim et al.[8]	PNET, mature cystic teratom	27	Early stage	Fertility preserving surgery+chemotherapy	30 months	-
Muhlstein et al.[9] Neuroblastoma 17 Early stage Surgery + chemotherapy					6 years	-
Lawlor et al.[10]	Neuroblastoma	13	Advanced (3c)	Surgery + chemotherapy	18 months	-
Ateşer et al.[11]	PNET	28	Advanced	Surgery + chemotherapy	13 months	+
Block et al.[17]	Recurrent metastatic ovarian neuroblastoma			Chemoradiotherapy	18 months	
Demirtaş et al.[18]	PNET	25	Early stage	Surgery + chemotherapy	3 years	+
Clinkard et al.[19]	Medulloblasto ma	23	Advanced	Surgery + chemotherapy	6 years	-
Ostwal et al.[20]	PNET	28	Advanced	Surgery + chemotherapy	18 months	+, pelvis
Kim et al.[21]	PNET	18	Advanced	Surgery + chemoradiotherapy	10 months, death	LN+, femur
Kleinman et al.[22]	PNET (25)	6-69	Early to advanced	Surgery + chemotherapy?/ radiotherapy	Max 9 years	
	-Differential (6)		2/6 stage 1 1/6 stage 2A 1/6 stage 3 1/6 stage?		4-5 years 3 years 5 years ?	
	-Primitive (12)		3/12 stage 1 6/12 stage 3		7 months/ 3y/9y 2-20 y 4 months	
	-Anaplastic (7)		1/7 stage 1 1/7 stage 2A 5/7		5 years ?	
Our study	PNET	64	Advanced	Surgery + chemoradiotherapy	7 months	

PNET: Primitive neuroectodermal tumors, LN: Lymph node, BSO: Bilateral salpingo-oophorectomy

Table 2. Immunohystochemical staining for ovarian primitive neuroectodermal tumors

	CD-99	SYNP	NSE	VIM	S100	GFAP	DESM	NF	CD-5 6	CD-3	CROMOGR	CYTOKE
Winkler et al.[6]	+/-	+	+	ND	+	+	ND	ND	ND	ND	-	ND
Yousefi et al.[7]	+	ND	-	+	ND	ND	-	ND	ND	ND	-	ND
Lim et al.(8)	-	+	ND	ND	ND	ND	-	ND	ND	+	ND	ND
Muhlstei n e al.[9]	-	+	+	ND	ND	ND	ND	ND	+	-	ND	+
Lawlor et al.[10]	ND	+	+	ND	ND	ND	-	+	ND	ND	ND	ND
Ateşer et al.[11]	+	-	ND	ND	-	ND	-	ND	ND	ND	ND	ND
Hirose et al.[12]	-	+	ND	ND	+	+	ND	ND	ND	+	ND	ND
Kuk et al.[13]	+	+	ND	+	+	+	-	ND	ND	ND	ND	-
Chu et al.[14]	-	+	ND	ND	+	+	ND	ND	+	ND	+	-
Lin et al.[15]	ND	ND	+	+	-	+	ND	+	ND	ND	ND	ND
Ostwal et al.[20]	+	-	ND	+	ND	ND	-	+	ND	ND	ND	ND
Our study	-	+	+	ND	-	ND	ND	-	ND	+	-	+
Xiao[24]	+	+	+	+	ND	ND	-	ND	ND	ND	ND	ND

PNET: Primitive neuroectodermal tumors, SYNP: Synaptophysin, NSE: Neuron spesific enolase, VIM: Vimentin, S100: Serum S100 protein, GFAP: Glial fibrillary acidic protein, DESM: Desmin, NF: Neurofilament protein, CD-56: Neural cell adhesion molecule, CROMOGR: Cromogranine, CYTOKE: Cytokeratin, ND: Not defined

surgery, patients received chemotherapy and/or radiotherapy. Although different chemotherapy agents were used for each patient in literature, generally therapies were designed as platinum-based[2,6,10,11,15,23]. Additionally ifosfamide, bleomycin, vincristine and doxorubicin, alternatively dacarbazine,[6] and adriamycin[5] were administered in some cases. The effect of radiotherapy has not been proved so there is need for more studies of cases of primary ovarian ES treatment. In the pathophysiologic pathway, the immature precursors of neural and glial cells from the embryonic period may proliferate and implant on the peritoneum and behave as malignant cells. However, other germ cells may persist and continue forming neural tube-like rosettes and medullary structures. All these stem from precursors in the neuroectoderm, and they are all called neuroectodermal tumors of the ovary. The translocation between chromosomes 11 and 22-t(11;22) (q24;q12)-is the same genetic problem for the PNETs group[8-10]. The differential diagnoses of PNETs of the ovary include several primary and metastatic ovarian neoplasms such as juvenile granulosa cell tumors, lymphoblastic lymphoma (LBL), extrauterine endometrial stroll sarcoma, and serous ovarian carcinomas. The distinction between ES of the ovary and other tumors is made through immunohistochemistry studies. As seen in Table 2,[12-15,24] on immunohistochemistry, diffuse membranous positivity for MIC2 (CD99), CD56 (neural cell adhesion molecule), HMW CK and FL1 led to the consideration of PNETs. Negativity for epithelial markers such as CK, EMA, desmin, and WT-1 led to the consideration of desmoplastic small round cell tumors (SRCTs). Positive staining of CD10, actin, and vimentin is considered as extrauterine stromal sarcoma, and negative staining is for PNET. Also, granulosa cell tumors are frequently reactive for CK and inhibit, although PNETs are non-reactive[25]. In immunohistochemistry, these tumors usually exhibit positivity for CD99, vimentin, and FLI-1. However, expression of many other markers can be found

including NSE, SYNP, chromogranin, CD56, CD57, S-100, and neurofilament protein. In addition, some tumors have focal positivity for CK. CD99 is a sensitive marker for PNETs, but also positive for some other SRCTs such as lymphomas, rhabdomyosarcomas. Accordingly, these findings limit the specificity of this antibody. As in our case, CD99 may be negative in 10% of tumors[26]. The differential diagnosis is broad and includes neoplasms composed of "small blue round" cells, which can be encountered in the ovary; small cell carcinoma of hypercalcemic type; extrauterine endometrial stromal sarcoma; rhabdomyosarcoma; melanoma; desmoplastic round cell tumor; and lymphoma/leukemia. Small cell carcinoma of hypercalcemic type affects adolescents and young adults, typically between the ages of 9 and 43 years and is associated with hypercalcemia[27]. Extrauterine endometrial stromal sarcomas are typically positive for vimentin and smooth muscle actin, and most tumors stain for CD10. Our tumor was negative for CD10. Embryonal rhabdomyosarcomas may be CD99 and FLI-1-positive[28]. It is characterized by alternating hyper and hypocellular myxoid areas and shows small cells admixed with spindle cells that may contain cross striations. There were no areas like those described above in our tumor and also myogenin was negative in our tumor[29]. Melanoma may be composed of small cells but it often arises in association with ovarian cystic teratomas. It may show melamine pigment and more conventional areas. The characteristic histologic appearance of desmoplastic round cell tumors is peripheral palisading of basaloid cells, forming irregular islands that may show central necrosis, surrounded by a desmoplastic stroma. Expression of keratins and destine may be helpful in the differential diagnosis. Expression of FLI-1 by LBL might potentially lead to a misdiagnosis of LBL as ES/PNET because we found diffuse FLI-1 positivity; but even when growing in a diffuse pattern, lymphomas still may show admixture of lymphoid and myeloid cells in different stages of maturation[28]. Also CD45 was negative in our case. There was a relation between disease stage and survival as shown in Table 1. However, one reported case in which the patient was young and had advanced stage at diagnosis had 6 years' disease-free survival after debulking surgery and adjuvant chemotherapy. Our patient had advanced stage disease and 7 months' disease-free survival after completing the therapy. In conclusion, preoperative findings and survival results of ovarian ES may be similar to epithelial ovarian cancer. However, treatment of these tumors is not standardized due to their rarity.

Ethics

Informed consent: Consent form was filled out by the patient.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.O., S.T., Concept: F.O., S.T., B.Y., Design: S.T., B.Y., F.O. Data Collection or Processing: B.Y., C.C.E Analyses or Interpretation: B.Y., C.C.E, S.T, Literature Search: B.Y., C.C.E Writing: B.Y., S.T., C.C.E., F.O.

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