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- All author name(s), institutional, corporate, or commercial affiliations, and up to two major degree(s).

- Corresponding author's name, address, telephone (including the mobile phone number), fax numbers and e-mail address (the corresponding author will be responsible for all correspondence and other matters relating to the manuscript).

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The precis is a one-sentence synopsis of no more than 30 words that describes the basic findings of the article. Precis sample can be seen below:

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Abstract

All manuscripts should be accompanied by an abstract. All information in the abstract should be consistent with the information in the text, tables, or figures. Avoid use of commercial names in the abstract. Original research reports should have a structured abstract of no more than 250 words, using the following headings:

· Objective: Main question, objective, or hypothesis (single phrase starting with, for example, "To evaluate..." or "To estimate." [never start with "To determine."]).

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· Results: Measurements expressed in absolute numbers and percentages, and when appropriate indicate relative risks or odds ratios with confidence intervals and level of statistical significance; any results contained in the abstract should also be presented in the body of the manuscript, tables, or figures.

· Conclusion: Directly supported by data, along with clinical implications.

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Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	5,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	2,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	3,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background. Avoid a detailed literature review in this section.

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Describe the research methodology (the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed) in sufficient detail so that others could duplicate the work. Identify methods of statistical analysis and when appropriate, state the basis (including alpha and beta error estimates) for their selection. Cite any statistical software programs used in the text. Express p values to no more than two decimal places. Indicate your study's power to detect statistical difference.

Address "IRB" issues and participants informed consent as stated above, the complete name of the IRB should be provided in the manuscript. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report

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outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNTh) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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The conclusion of the study should be highlighted. The study's new and important findings should be highlighted and interpreted.

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Clinical Investigation / Araştırma

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Endometrial CD56+ natural killer cells in women with recurrent implantation failure: An immunohistochemical study

Tekrarlayan implantasyon başarısızlıklarında endometriyal CD56+ natürel killer hücreleri: İmmünohistokimyasal çalışma

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Abstract

Objective: Implantation failure is a multifactorial problem of reproductive medicine. However, the mechanism of this process is still not fully understood. There is increasing evidence that these cases of recurrent implantation failure might have an immunologic background. Uterine natural killer (NK) cells provide immune-modulation at the interface between maternal decidua and the trophoblast. The aim of this study to evaluate whether there was a significant difference in the number of endometrial CD56+ NK between women with a history of recurrent implantation failure and women who had a live birth.

Materials and Methods: Patients with a history of recurrent implantation failure were included in the study. Twenty-five women with a history of recurrent implantation failure were assigned to the case group, and 25 women who had one or more live births were assigned to the control group. Endometrial biopsies were obtained during the luteal phase on the 21st-24th day of the menstrual cycle.

Results: There was a statistically significant difference between the groups concerning the number of deliveries ($p<0.001$) and miscarriages ($p<0.001$). The mean number of uNK was 10.5±10.5 cells/mm^2 in the case group and 19.2±11.2 cells/mm^2 in the control group. There was a statistically significant difference between the two groups ($p=0.003$).

Conclusion: Implantation failure is a multifactorial problem of reproductive medicine. The results of our study suggest that uterine NK play a role in the progress of normal pregnancy and reduced uterine NK cell numbers were associated with implantation failure.

Keywords: CD56, immunohistochemistry, *in vitro* fertilization, recurrent implantation failure, uterine natural killer cells

Öz

Amaç: İmplantasyon başarısızlıkları reprodüktif tıbbın multifaktöriyel bir problemidir. Tam mekanizması günümüzde halen aydınlatılamamıştır. Son yıllarda mekanizmasında immünolojik faktörlerin etkili olduğuna dair kanıtlar artmaktadır. Uterin natürel killer (uNK) hücreleri trofoblastlar ile desidua arasında immün modülasyonu sağlar. Çalışmamızın amacı tekrarlayan implantasyon başarısızlığı olan olgular ile canlı doğuma sahip olan kadınlar arasında uNK hücreleri açısından fark olup olmadığının araştırılmasıdır.

Gereç ve Yöntemler: Çalışmaya tekrarlayan implantasyon başarısızlığı olan hastalar dahil edildi. Yirmi beş implantasyon başarısızlığı olan hasta olgu grubunu, 25 canlı doğum öyküsü olan kadın ise kontrol grubunu oluşturdu. Endometriyal biyopsiler menstruel döngünün 21. ile 24. günleri arasında luteal fazda alındı. Her iki grup uNK açısından karşılaştırıldı.

Bulgular: Her iki grup arasında canlı doğum ($p<0,001$) ve düşük sayısı ($p<0,001$) açısından anlamlı fark vardı. Olgu grubunda ortalama uNK sayısı 10,5±10,5 cells/mm^2 idi. Kontrol grubunda ise 19,2±11,2 cells/mm^2 idi. Olgu grubunda uNK sayısı anlamlı olarak daha az olarak bulundu ($p=0,003$).

Sonuç: İmplantasyon başarısızlıkları reprodüktif tıbbın multifaktöriyel bir problemidir. Çalışmamızda uNK hücrelerinin gebeliğin devamında önemli bir rol oynadığı ve sayılarının azalmasının tekrarlayan implantasyon başarısızlığıyla ilişkili olduğu bulunmuştur.

Anahtar Kelimeler: CD56, immünohistokimya, *in vitro* fertilizasyon, tekrarlayan implantasyon başarısızlığı, uterin naturel killer hücreleri

PRECIS: We evaluated whether there was an effect of the number of endometrial CD56+ NK on women with a history of recurrent implantation failure.

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Introduction

Accomplished implantation of an eight-cell embryo into the endometrium is mandatory for reproduction. The rate of successful implantation of an embryo is approximately 30%[1]. Implantation failure is a multifactorial problem of reproductive medicine. Recurrent implantation failure (RIF) was defined as a failure of pregnancy after at least three previous assisted reproductive technique cycles, or implantation failure with a transfer of more than four embryos by the European Society of Human Reproduction and Embryology. A successful pregnancy requires synchronization between the trophoblasts and endometrium. However, the mechanism of this process is still not fully understood.

There is increasing evidence that these cases of RIF might have an immunologic background. The endometrium plays a role in implantation physiology via immune cells, cytokines, and chemokines[2]. Multiple kinds of immune cells are potentially involved in supporting immune tolerance during implantation and successful ongoing pregnancy[3]. Uterine natural killer (uNK) cells express their specific cell surface marker CD56, and differ from blood NK cells[4]. These uNK cells are the dominant leukocyte population (70-90% of uterine lymphocytes) in the decidua at the time of implantation and early placentation[5]. Although the function of uNK cells is uncertain, the regulation of uNK cells at the time of eight-cell embryo implantation is thought to feature in implantation. In a normal pregnancy, uNK cells provide immune-modulation at the interface between decidual tissue and trophoblast. We aimed to evaluate whether there was a significant difference in the count of endometrial CD56+ NK between women with RIF and women who had a live birth.

Materials and Methods

Participants and Study Design

Twenty-five women with RIF and 25 women who had one or more live births between January 2012 and December 2017 were included in the study. RIF was defined as the failure of good quality embryos to implant after at least 3 cycles of IVF. Women with positive for anti-phospholipid antibodies (ANA, anticardiolipin IgM and IgG, anti-DNA, antiphospholipid IgM and IgG), anti-toxoplasma IgM, and/or anti-rubella IgM were excluded from the study. Women with abnormal thyroid function tests results, anti-thrombin III deficiency, protein C or S deficiency, factor-V-Leiden mutation, prothrombin gene mutation, mutation of *MTHFR C677T* gene, and/or mutation of *MTHFR A1298C* gene were also excluded from the study. Hysterosalpingography was performed in all infertile patients before the procedure and there were no abnormal findings. Twenty-five women who had one or more live births were assigned to the control group. None of the 25 women in the control group received assisted reproduction treatment at any time. The local ethics committee approved the study (approval no: 20181146). The patients were informed orally and in writing.

Collection of Tissue Samples and Histopathologic Examination

Endometrial biopsies were obtained during the luteal phase on the 21st-24th day of the menstrual cycle using the Pipelle device and fixed in 10% formaldehyde. Formalin-fixed and paraffin-embedded tissue samples were incubated for 120-minutes with the CD 56 primary antibody (NCL-L-CD56-1B6, 1/200 dilution, Novocastra Laboratories Ltd.), 30 minutes with biotin (Dako LSAB System-HRP, Dako North America, Inc., K0690), and 30 minutes with streptavidin (Dako LSAB System-HRP, Dako North America, Inc., K0690), respectively. Aminoethylcarbazole chromogen was added for 15 minutes. Paraffin-embedded tissue samples were stained using Mayer's hematoxylin. The same pathologist evaluated all samples using an Olympus BX53 microscope at 400x magnification. CD56+ cell counts were determined as cells/mm^2 (Figure 1).

Statistical Analysis

Data were analyzed using the SPSS software package (22.0, IBM SPSS Statistics for Windows; IBM Corp. Armonk, NY). Histogram, normality plots, and thre Shapiro-Wilk normality test were used to analyze data distribution. Descriptive statistics (mean, standard deviation, median, range, percentage) were used in the analysis of quantitative data. The chi-square (χ^2) test or Fisher's Exact test was used to analyze qualitative data. The Mann-Whitney U test was used in the analysis of quantitative data. Statistical significance was established at $p<0.05$.

Results

Twenty-five women with RIF and 25 women as fertile controls were included in the study. The parental chromosomes were normal in all women. The mean ages of the case and control groups were 33.5±5.6 and 34.4±5.3 years, respectively. The two groups were similar in terms of age (p=0.224). There were statistically significant differences between the groups in

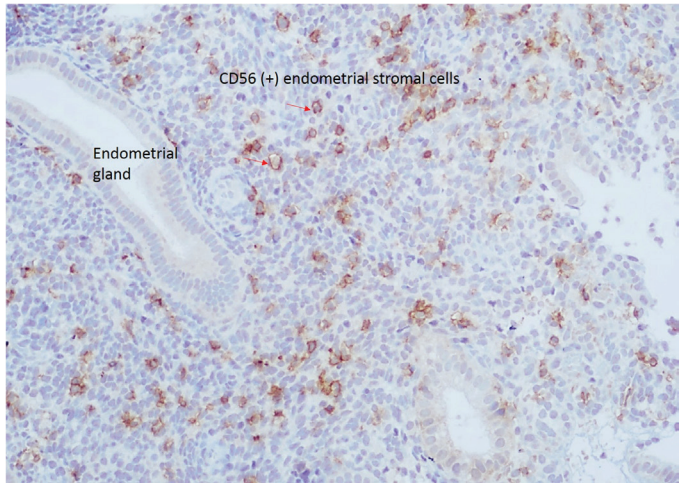

Figure 1. CD56+ cells were shown in the endometrial tissue

terms of the number of deliveries (p<0.001) and miscarriages (p<0.001) (Table 1). The mean number of uNK was 10.5±10.5 cells/mm^2 in the case group and 19.2±11.2 cells/mm^2 in the control group. There was a statistically significant difference between the two groups in terms of uterine NK (p=0.003) (Table 1). There was a significant positive correlation between the number of uNK cells and the number of miscarriages (r=0.430) (p=0.002). There was also a significant positive correlation between the number of uNKs and the number of live birth (r=0.463) (p=0.001). Correlation coefficients of parity, live birth, miscarriages, and uNK of the patient group are shown in Table 2.

Discussion

The endometrial leukocyte population consists of T-cells, macrophages, and natural killer cells. T-cells constitute 45% of leukocytes in the proliferative phase. Although their numbers remain constant throughout the cycle, their rate in proliferative phase is higher compared with other types of leukocytes. The most important leukocyte population in the endometrium comprises uNK cells. These lymphocytes contain the NK cell surface antigen CD56. During the implantation, large granular lymphocytes constitute 70-80% of the leukocyte population, and if conception occurs, their number increases more. UNK cells are particularly abundant in the uterus at the time of implantation and are in close contact with placental trophoblast cells. UNK cells have a major role in both implantation and the development of the placenta and vascularization. It limits the trophoblast invasion to decidua. In the absence of implantation, uNK cells undergo apoptosis and are therefore thought to play a role in the initiation of menstrual bleeding. UNK cells also secrete several growth factors involved in angiogenesis, such as VEGF, placental growth factor, and angiopoietin-2.

Giuliani et al.[6] found that the number of endometrial CD56+ cells was not significantly different in women with recurrent pregnancy loss. By contrast, Quenby et al.[7] demonstrated that the mean count of uNK cells was significantly higher in women with recurrent pregnancy loss than women with had a live birth history. Clifford et al.[8] also described increased expression of uNK in 29 women with recurrent pregnancy loss. Similarly, in the current study, there was a positive correlation between the number of miscarriages and the amount of endometrial CD56+ cells. There was also a positive correlation between the number of live births and the number of endometrial CD56+ cells. Different etiologies except for reduced uNK, such as chromosomal abnormalities, could be a reason for miscarriage. Sacks and Finkelstein[9] found that uNK numbers increased dramatically from about 5% of stromal cells in the follicular and early luteal phases of the menstrual cycle to 30-40% of stromal cells in the mid and late luteal phases when implantation occurred. Gaynor and Colucci[10] indicated that uNK numbers increased further to as much as 70% of stromal cells if implantation occurred. The current study demonstrated that there was a positive correlation between the number of live births and the number of endometrial CD56+ cells, and there was also a significantly reduced density of CD56+ cells in women with recurrent implantation failure. In contrast, Tuckerman et al.[11] found that the high density of CD56+ cells in the endometrium of women with RIF was directly involved in implantation duration. Additionally, Santillan et al.[12] found a higher density of endometrial CD56+ cells in women with RIF than in controls. They suggested that testing for endometrial NK cells might be helpful in women with idiopathic RIF during the luteal phase.

We acknowledge that the small sample size, retrospective nature, and lack of the chromosome analysis of the miscarriage tissues are the main limitations of the study. Increasing the number of patients and including other subgroups of CD 56+ cells to the study may help explain the mechanism.

Table 1. Patient demographics and comparison of the number of endometrial CD56+ NK between groups

	Implantation failure (n=25)	Controls (n=25)	p
Age (years)	33.5±5.6	34.4±5.3	0.224
No. of deliveries	0	2 (1-7)	**<0.001**
No. of miscarriages	0	2.0 (1-3)	**<0.001**
uNK	10.5±10.5	19.2±11.2	**0.003**

uNK: Uterine natural killer

Table 2. Correlation coefficients of parity, live births, miscarriages, and uNK of the patient group

	Parity		Live birth		Miscarriages		uNK	
	r	p	r	p	r	p	r	p
Parity	1	<0.001	0.870	<0.001	0.963	<0.001	0.476	<0.001
Live birth	0.870	<0.001	1.000	<0.001	0.827	<0.001	0.463	0.001
Miscarriages	0.963	<0.001	0.827	<0.001	1	<0.001	0.430	0.002
uNK	0.476	<0.001	0.463	0.001	0.430	0.002	1	<0.001

uNK: Uterine natural killer

Conclusion

Implantation failure is a multifactorial problem of reproductive medicine. However, the mechanism of this process is still not fully understood. The results of our study suggest that uNKs play a role in the progress of normal pregnancy and reduced uNK cell numbers were associated with implantation failure. We believe that further studies will explain the role of these cells in the etiology.

Ethics

Ethics Committee Approval: The local ethics committee approved the study (approval no: 20181146).
Informed Consent: The patients were informed orally and in writing.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.G., P.O., M.C.Ç., Design: Y.E.P., R.A., M.C.Ç., Data Collection or Processing: P.O., R.A., Analysis or Interpretation: P.O., R.A., Literature Search: P.O., R.A., Writing: Y.E.P., B.G., R.A., G.B.
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Comparison of intramuscular versus subcutaneous aqueous progesterone for luteal phase support in artificially prepared frozen embryo transfer cycles

Hormon replasmanı ile hazırlanmış dondurulmuş embriyo transfer sikluslarında intramusküler progesteron ve su bazlı subkütanöz progesteronun luteal faz desteği açısından karşılaştırılması

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Abstract

Objective: Cryopreservation of embryos for future transfer attempts has noticeably increased in the last decade, especially due to the technological developments in *in vitro* fertlization (IVF) laboratories. In parallel, different progesterone (P) replacement regimens preceding artificially prepared frozen embryo transfer (AC-FET) attempts, especially with respect to the route of application and dosing scheme, have been widely argued so far. We aimed to provide more information about the efficacy profile of novel subcutaneous aqueous progesterone (SP) in AC-FET cycles.

Materials and Methods: This retrospective, single-centre cohort study included a total of 507 AC-FET cycles performed between June 2018 and April 2020. Three hundred forty-nine (68.8%) patients received 50 mg of intramuscular progesterone as once daily, 158 (31.2%) patients received 25 mg of SP as twice daily. Only, the first and single blastocyst transfers from the same cohort were accepted. The inclusion criteria were as follows: females aged <37 years, body mass index ≥18 kg/m^2 and ≤35 kg/m^2, sperm concentration ≥5x10^6/mL. Pre-implantation genetic testing cycles were not included. The primary outcome was the live birth rate (LBR).

Results: The number of previous IVF attempts, type of infertility, peak estradiol (E2) levels, the total number of retrieved oocytes, mature oocytes, and the number of 2PN was significantly different between the groups. Positive pregnancy (p=0.474) and clinical pregnancy rates (p=0.979), LBR (p=0.404), and missed abortion rates (p=0.144) were comparable between the groups. The total number of oocytes [adjusted odds ratios (AOR)=1.024, 95% confidence interval (CI): 1.002-1.047; p=0.03)], endometrial thickness (AOR=1.121, 95% CI: 1.003-1.253; p=0.044), and cryopreservation day 5/6 (AOR=0.421, 95% CI: 0.226-0.788; p=0.007) achieved statistical significance following binary logistic regression analysis. However, P administration type did not achieve statistical significance (p=0.731).

Conclusion: As a novel option, SP has comparable efficacy in pregnancy outcomes and may be accepted as an alternative for luteal phase support in AC-FET cycles.

Keywords: Subcutaneous aqueous progesterone, intramuscular progesterone, artificially prepared frozen embryo transfer

Öz

Amaç: Son on yılda, özellikle *in vitro* fertilizasyon (IVF) laboratuvarında meydana gelen teknolojik gelişmeleri takiben, embriyo kriyoprezervasyonu ve bu embriyoların gelecekteki transfer işlem sayıları fark edilir bir şekilde artmıştır. Buna paralel olarak, hormon replasmanı ile hazırlanmış dondurulmuş-çözülmüş embriyo transferi (HR-DÇET) öncesi uygulanan progesteron (P) yerine koyma tedavileri de, özellikle uygulama yolu ve dozları açısından daha çok tartışılmaya başlanmıştır. Bu çalışmada, yeni bir formülasyon olan su bazlı subkütanöz progesteronun (SP) HR-DÇET tedavilerindeki etkinliği hakkında daha fazla bilgi edinmeyi amaçladık.

PRECIS: Subcutaneous aqueous progesterone is an effective alternative to intramuscular progesterone in artificially prepared frozen embryo transfer cycles.

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Gereç ve Yöntemler: Retrospektif, tek merkezli kohort çalışması olarak dizayn edilen bu çalışma, Haziran 2018 ve Nisan 2020 tarihleri arasında yapılan 507 tane HR-DÇET siklusunu içermektedir. Hastaların 349'unda (%68,8) 50 mg intramusküler progesteron günde bir defa, 158'inde (%32,2) ise 25 mg SP günde iki defa kullanıldı. Aynı tedavi siklusunda elde edilen ve dondurulan blastokistlerden sadece bir tanesinin transferi değerlendirmeye alındı. Aynı hastanın tekrarlayan tedavi siklusları çalışmaya dahil edilmedi. Dahil edilme kriterleri; kadın yaşı <37, vücut kitle indeksi ≥18 kg/m^2 ve ≤35 kg/m^2, sperm konsantrasyonu ≥5x10^6/mL, olarak kabul edildi. Preimplantasyon genetik tanı uygulanan sikluslar değerlendirme dışı bırakıldı. Birincil olarak canlı doğum oranları (LBR) ele alındı.
Bulgular: Önceki IVF deneme sayısı, infertilite tipi, zirve serum estradiol (E2) değerleri, toplanan oosit sayısı, metafaz II ve 2PN sayıları gruplarda anlamlı farklılık gösterdi. Pozitif gebelik (p=0,474), klinik gebelik (p=0,979) ve LBR (p=0,404) ve aynı zamanda bozulmuş gebelik oranları (p=0,144) gruplarda benzerdi. İkili lojistik regresyon analizinde toplanan oosit sayısı [ayarlanmış risk oranı (AOR)=1,024, %95 güven aralığı (CI): 1,002-1,047; p=0,03)], endometriyal kalınlık (AOR=1,121, %95 CI: 1,003-1,253; p=0,044) ve kriyoprezervasyon günü (AOR=0,421, %95 CI: 0,226-0,788; p=0,007) istatistiksel olarak anlamlı bulundu. Fakat, kullanılan P tipi LBR sonuçlarında anlamlı bir fark yaratmadı (p=0,731).
Sonuç: SP enjeksiyonları gebelik sonuçları üzerinde karşılaştırılabilir bir etkiye sahiptir ve HR-DÇET sikluslarında alternatif bir tedavi olarak kabul edilebilir.
Anahtar Kelimeler: Subkütanöz progesteron, intramüsküler progesteron, dondurulmuş-çözülmüş embriyo transferi

Introduction

Almost 37 years ago, the first human pregnancy was reported following frozen embryo transfer (FET)[1]. Following the developments in the *in vitro* fertilization (IVF) field, the cryopreservation of embryos and subsequent FET strategy has doubled in the last decade[2]. Artificial endometrial preparation is one of the methods used for FET cycles and has been found as successful as the other approaches[3]. In these cycles, minimal monitoring is required, and the timing of embryo transfer and initiation of progesterone (P) is more flexible[4]. Therefore, it allows both the physicians and embryology staff to easily organize daily business planning.

Exogeneous P replacement is preceded by estrogen supplementation and its use is mandatory to prepare the endometrium for successful implantation and the survival of the pregnancy[5]. Exogeneous P can be administered by different routes: intramuscular, vaginal, oral, rectal, and recently, subcutaneous. Oral micronised P formulations are exposed to the first-pass effect within the liver, hence they have a low effect profile[6]. Vaginal formulations such as capsules, gels or suppositories showed a similar efficacy profile when compared with each other or by the intramuscular route[7-9]. However, debates regarding the method of application, the timing for luteal phase support (LPS), and doses are ongoing[10]. Oil-based intramuscular progesterone (IMP) preparations are painful and may cause serious adverse effects such as skin inflammation and sterile abscesses, but they have been found to decrease subendometrial uterine contractility better than vaginal progesterone (VP), and this positive effect has been related to increased pregnancy outcomes and decreased rates of embryo displacement following the attachment process[11].

In the light of new technological developments, subcutaneous aqueous progesterone (SP) has gained a more hydro-soluble and absorbable state by the addition of β-cyclodextrin[12]. Two randomized controlled trials (RCT) conducted on fresh transfer cycles compared the efficacy of SP and VP and reported similar ongoing pregnancy rates (OPRs) and live birth rates (LBRs) [13,14]. Regarding the degree of acceptance and satisfaction, the authors found significantly increased acceptance rates for the SP route compared with VP[15].

The use of SP continues to gain popularity in our daily practice. Today, most physicians use VP or IMP regimens, alone or in combination, for LPS. Currently, there are insufficient data on the effectiveness of the new formulation in artificially prepared FET (AC-FET) cycles. Therefore, in our study, we aimed to contribute to the literature by comparing two different P replacement regimens, SP vs. IMP, in elective, single blastocyst AC-FET cycles.

Materials and Methods

Design

In this retrospective, single-center cohort study, we reviewed the pregnancy outcomes of 507 AC-FET cycles, performed between June 2018 and April 2020 in Bahceci Fulya IVF centre. The reason for choosing the time interval in this way was the introduction of the SP formulation in Turkey in June 2018. Ethics approval was obtained from the institutional review board (approval number: 59, date: 21/03/2020). Based on our experience and reports in the literature, we switched to the freeze-all and subsequent FET strategy in all IVF cycles in 2013 due to its superior reproductive outcomes compared with fresh transfer[16,17]. Only the first single blastocyst transfers from the same cohort were included in the study. The eligibility criteria for the couples were as follows: female age <37 years, body mass index (BMI) ≥18 kg/m^2 and ≤35 kg/m^2, sperm concentration ≥5x10^6. Couples with a history of repeated implantation failure (>2), recurrent miscarriages (≥2), past surgery(-ies) for intrauterine adhesions, submucosal fibroids and mullerian anomalies (unicornuate, bicornuate, septate uterus) were excluded from the study. Also, couples carrying chromosomal abnormalities and preimplantation genetic testing cycles were not included.

Ovarian Stimulation

The gonadotropin-releasing hormone (GnRH) antagonist protocol was the preferred method for ovarian stimulation. On the 2nd or 3rd day of the menstrual cycle, gonadotrophin injections were started by using recombinant follicle-stimulating hormone (Gonal-F; Merck Serono, Geneva, Switzerland) and/or highly purified human menopause gonadotrophins (hp-hMG) (75-150 IU, Merional; IBSA) preparations. The dose regimens

were designated at the physician's preference. When the leading follicle exceeded 13 mm in diameter, 0.25 mg of GnRH antagonist (Cetrotide; Serono) was started daily until the day of maturation trigger. Maturation of the oocytes was induced either with the use of 250 µg of human chorionic gonadotropin (hCG; Ovitrelle, Serono) or 0.2 mg triptorelin (Gonapeptyl, Ferring). Transvaginal sonography (TV-USG)-guided oocyte retrieval was performed 35-36 hours later.

Laboratory Process

After the denudation process, each metaphase II oocyte was injected with sperm using the intracytoplasmic sperm injection technique and cultured individually in a special pre-equilibrated culture dish. A fertilization check was performed 16-18 hours after insemination. A single-step media (Irvine Scientific, CA, USA) was used throughout the blastocyst culture period. Blastocyst quality assessment was performed on day 5 or 6 by two senior embryologists, with the aid of a morphology-based three-part scoring system as described previously[18,19]. Once the embryo reached the expansion degree of at least 3, vitrification was performed for cryopreservation. Categorization of blastocysts was as follows: excellent (≥3 AA), good (3, 4, 5, or 6 and AB, AC, BA, BB), poor (3, 4, 5, or 6 and BC, CB, CC, or CA).

Artificial Preparation of FET Cycle

Endometrial preparation was started on day 2 or 3 of menstrual bleeding with estradiol valerate pills (Estrofem, Novo Nordisk, Denmark) at a dosage of 6 mg/day. A stable dosing scheme was implemented. Follow-up visits were performed between day 10 and 14 of treatment. Endometrial thickness was measured using TV-USG and blood was drawn to detect serum estradiol (E2) and P levels. The dosage of E2 pills was increased to 8 mg/day if the thickness was <7 mm and an additional follow-up visit was planned within the next seven days for confirmation. According to the patient's and physician's preference, LPS was initiated either with 50 mg IMP injection (Progestan, Kocak Farma, Turkey) once per day, or with 25 mg of SP (Prolutex, IBSA, Switzerland) injections, twice daily. The first dose of IMP was injected between 4 and 7 pm, and subsequent doses were repeated every 24 hours at the same time interval. For the SP injection, the first dose was injected between 8 am and 10 am, and the second dose was injected 12 hours later. The same scheme was followed every day. In our daily routine, all transfers are performed between 4 pm and 7 pm. Accordingly, FET was performed following the 5th dose of IMP and the 11th dose of SP administration. Serum β-hCG levels were measured 12 days after FET and levels ≥5 IU were accepted as positive. Afterwards, E2 replacement was stopped at the 6th week of pregnancy, whereas P was continued until 10 weeks in both arms.

Outcomes

Primary outcome was the LBR per embryo transfer. Clinical pregnancy (CP) was defined as the confirmation of an intrauterine gestational sac at 6-7 weeks of pregnancy. Missed abortion (MA) was defined as a CP loss before 20 weeks' gestation.

Statistical Analysis

For the first step, the Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to understand whether the continuous variables followed a normal distribution. Accordingly, the median (quartile 1- quartile 3) values of these variables were reported in the tables. Afterwards, the independent samples median test was run to determine if there were differences in continuous parameters between patients in the two treatment groups. The chi-square test was performed to test the significance of each categorical parameter and the results were reported as percentages.

A binary logistic regression model was performed regarding outcomes to determine whether a patient was having a live birth. In this model, female age, duration of infertility, sperm concentration, type of infertility, total number of retrieved oocytes, endometrial thickness, cryopreservation day (D5 or D6), blastocyst quality (excellent, good, poor), peak E2 levels in FET and type of P administration (IMP or SP) were allocated as independent variables. The backward conditional procedure was used and variables that were not statistically significant were removed from the model. The final binary logistic model reported only the statistically significant parameters. To measure the effect of each significant variable, both unadjusted and adjusted odds ratios were reported. Unadjusted odds ratios (UAOR) indicated the effect of each variable when all of the other factors were eliminated and only the specific variable was taken into consideration. Adjusted odds ratios (AOR) were calculated when all the significant independent variables were taken into account, simultaneously.

Results

All 507 patients in our study were assigned to one of the two LPS alternatives. IMP was used in 349 (68.8%) AC-FET cycles and SP was used in 158 (31.2%) AC-FET cycles. Two groups were matched concerning demographics and embryologic parameters as shown in Table 1. Accordingly, the median values of the number of previous IVF attempts, peak E2 levels, the total number of oocytes, mature oocytes, and the number of 2PN zygotes were significantly different between groups.

Table 2 displays the characteristics of AC-FET cycles and pregnancy outcomes. As shown, the only parameter to reach statistical significance was the peak E2 levels, which were measured on the day of or one day before the initiation of P replacement (p=0.025). There were no significant differences between groups, regarding positive pregnancy rates (p=0.474), CP rates (p=0.979), LBRs (p=0.404), and MA rates (p=0.144).

Binary logistic regression analysis was performed to determine the independent variables, those which had a significant effect on live birth outcome (Table 3). The final model was

Table 1. Demographics, clinical and embryologic parameters

	IMP (n=349)	SP (n=158)	p-value
Female age	30 (27-32)	29 (26-32)	0.305
BMI (kg/m^2)	24.11 (21.48-27.46)	22.86 (20.7-27.09)	0.073
Duration of infertility	3 (2-4)	3 (2-5)	0.913
Previous IVF attempts	0 (0-0)	0 (0-0)	0.003*
Sperm concentration (10^6/mL)	78 (39.8-135)	67.75 (30-121.25)	0.475
Type of infertility	132/349 (37.8)	47/158 (29.7)	0.017*
Female	62/349 (17.8)	38/158 (24.1)	
Male	135/349 (38.7)	71/158 (44.9)	
Unexplained (UEI)	20/349 (5.7)	2/158 (1.3)	
Combined			
Total dose of gonadotropins (IU)	2250 (1837.5-2700)	2250 (1931.25-2793.75)	0.850
Peak E2 levels (pg/mL)	1978 (1408.5-2962.5)	2710.5 (1754.75-4220.5)	<0.001*
Total number of oocytes	12 (9-20)	16 (11.75-22)	0.002*
No. of mature oocytes	10 (7-16)	13 (10-18.25)	0.003*
No. of 2PN	9 (6-13)	11 (7-15.25)	0.001*
Fertilization rate (FR)	84.21 (75-92.45)	83.33 (73.60-91.83)	0.843
Blastulation rate (per 2PN)	44.44 (32.22-60)	43.65 (29.64-64.39)	0.687

Values are reported as median (Q_1-Q_3).
Independent samples median test was used to test the median value between the IMP and SP groups.
*statistically significant p-value at the α=5% level, IMP: Intramuscular progesterone, BMI: Body mass index, IVF: *In vitro* fertlization

Table 2. Properties of FET cycles and pregnancy outcomes

	IMP	SP	p-value
Endometrial thickness (mm)	9.4 (8.3-10.65)	9.7 (8.88-11)	0.224
Cryopreservation day			
Day 5	317/349 (90.8)	143/158 (90.5)	0.907
Day 6	32/349 (9.2)	15/158 (9.5)	
Blastocyst Quality			
Excellent	131/349 (37.5)	65/158 (41.1)	0.301
Good	125/349 (35.8)	61/158 (38.6)	
Poor	93/349 (26.6)	32/158 (20.3)	
Peak E2 levels in FET (pg/mL)	228 (182-308.5)	253 (187.75-330.50)	0.025*
Positive pregnancy (%)	273/349 (78.2)	128/158 (81)	0.474
Clinical pregnancy (%)	250/349 (71.6)	113/158 (71.5)	0.979
Missed Abortus (%)	45/273 (16.5)	14/128 (10.9)	0.144
Live birth (%)	205/349 (58.7)	99/158 (62.7)	0.404

χ^2 test was used to test the proportions between the IMP and SP groups for categorical variables.
*statistically significant p-value at the α=5% level.
Independent samples median test was used to test the median value for continuous values, FET: Frozen embryo transfer, SP: Subcutaneous aqueous progesterone, IMP: Intramuscular progesterone

statistically significant, χ^2 (2)=18.373, p<0.001. The model explained 4.8% (Nagelkerke R^2) of the variance in live births and correctly classified 62.1% of cases. As shown in Table 3, UAOR and AOR concluded that variables such as the total

Table 3. Logistic regression model on live birth outcome

					95% CI for OR	
	B	Wald	p-value	OR	Lower	Upper
Total no. of oocytes	0.024	4.715	0.030	1.024	1.002	1.047
Endometrial thickness	0.114	4.040	0.044	1.121	1.003	1.253
Cryopreservation day						
Day 5	Reference	----	----	-----	-----	-----
Day 6	-0.864	7.338	0.007	0.421	0.226	0.788

A binary logistic regression model was used with a backward conditional procedure. The outcome variable was taken as having a live birth or not. References category on the cryopreservation day was taken as day 5.
*statistically significant p-value at the α=5% level, CI: Confidence interval, OR: Odds ratio

number of retrieved oocytes, endometrial thickness, and cryopreservation day were statistically significant both when considered separately and when taken into analysis at the same time. AC-FET cycles using day 6 cryopreserved blastocysts resulted in a 57.9% less likely live births compared with day 5 blastocyst transfers [(AOR=0.421, 95% confidence interval (CI): 0.226-0.788; p=0.007)]. It is also shown that when the total number of retrieved oocytes increased by one unit, patients were 1.024 times more likely to have a live birth, and similarly, when the endometrial thickness increased by one unit, patients were 1.121 times more likely to have a live birth (AOR=1.024, 95% CI: 1.002-1.047; p=0.03 and AOR=1.121, 95% CI: 1.003-1.253; p=0.044, respectively). P administration type did not achieve statistical significance (p=0.731).

Discussion

As far as we know, this is the first study to compare the clinical efficiency profiles of the novel aqueous SP formulation and IMP in AC-FET cycles. The results of our study showed non-inferior pregnancy outcomes of 50 mg daily SP administration in women undergoing AC-FET compared with IMP.

For many years, owing to its insoluble properties, the only way to administer the synthetic progesterone hormone was through intramuscular injections. Although it has many adverse effects and causes discomfort, most studies used IMP as a reference when comparing other formulations due to its reliable contributions to pregnancy outcomes[20-23]. The aim of producing a new injectable P formulation was to provide the advantage of existing parenteral injection on pregnancy results, and to eliminate its adverse effects, complications, and negative effects on patient comfort[24,25]. For this purpose, Sator et al.[26] assessed the bioavailability of the novel SP formulation in comparison with oil-based IMP among postmenopausal and reproductive-aged women. Irrespective of the route of administration (i.m. and s.c.), serum maximum concentrations (C*max*) of SP product were 3-4 times higher than the C*max* of the oily IMP (p<0.001). Moreover, T*max* (time to achieve C*max*) was 7 times shorter in the SP group. Regarding the safety profiles, the authors reported lower frequency and shorter duration of adverse effects, those related to hormonal changes and injection site reactions. In another valuable study, histologic changes caused by two different dosing regimens, 25 mg/daily and 50 mg/daily, of SP were investigated via endometrial sampling[27]. The authors reported adequate pre-decidual transformation within the endometrial specimens of the entire cohort and concluded that the new formulation was a valid option for LPS. From the clinical point of view, the narrow BMI range (>19 and <25 kg/m^2) in the study should be interpreted with caution and further well-designed studies could give more accurate information, especially in overweight and obese women.

There is still no consensus on the best route of P administration for replacement in AC-FET cycles. According to a Cochrane review, there was no significant difference between VP and IMP in terms of CP, MA rates, and LBRs[28]. However, the authors declared that the results were insufficient to draw a definite conclusion due to the heterogeneity between the included studies. In a more recent analysis in which VP and IMP were compared in FET cycles, similar pregnancy outcomes were reported[8,29]. By contrast, Devine et al.[30] reported decreased OPRs only in the VP group when compared with VP plus IMP and IMP only, and they terminated the randomization arm due to increased SA rates (47%, 30%, and 23%, respectively, p<0.001). The broad range of age selection criteria (18-48 years) and the nine-day use of VP before FET should be taken into account. In another study, significantly lower rates of CP and live births were reported in the VP group following day 3 FET.

The main limitations of the study were the use of the slow freezing technique for cryopreservation and the 3rd day embryo transfers instead of blastocyst-stage transfers. Similar to the inconsistent results mentioned in the above studies, using oral dydrogesterone for FET cycles also needs further investigation[31,32].

As all IVF practitioners know, daily gonadotropin injections are made throughout the stimulation phase of IVF treatments. Therefore, patients are familiar with subcutaneous injection attempts and feel safe while self-administering SP[12]. Moreover,

the lesser injection site pain is an advantage of SP, probably related to its water-soluble content[15]. Another advantage of SP use is preventing the messy discharge reported with VP application.

Study Limitations

The major weaknesses of our study are its retrospective design and lack of randomization for the type of P formulations. Its retrospective nature is also the greatest obstacle to reaching information about patient comfort. The main reason for the small sample size is that the SP form started to be used in our country approximately two years ago. We designed this study in patients who were aged younger than 37 years to alleviate the risk of aneuploidy, which might give rise to increased rates of abortions. Four hundred eighty out of 507 (94.6%) patients in the study were aged younger than 37 years. Due to the legal restrictions in our country, we included only single blastocyst transfers. We believe that the strict inclusion and exclusion criteria helped us to generate homogenous groups and detailed analysis of the variables added strength to our work.

Conclusion

This study provides clinical evidence that the newly developed SP formulation has a comparable efficiency profile on pregnancy outcomes and is a strong candidate for LPS in AC-FET cycles. Future prospective studies and RCT are needed to clarify the best way regarding various P replacement regimens.

Ethics

Ethics Committee Approval: Ethics approval was obtained from the institutional review board (approval number: 59, date: 21/03/2020).
Informed Consent: Retrospective study.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions:

Surgical and Medical Practices: M.B., Concept: F.K.B., Design: M.B., Data Collection or Processing: Z.Y., M.G., E.N.T., Analysis or Interpretation: Z.Y., Literature Search: E.N.T., Writing: E.N.T.
Conflict of Interest: The authors report no conflict of interest.
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In vitro maturation with letrozole priming: Can it be a solution for patients with cancerophobia? A pilot study

Letrozol ile öncüllenmiş in vitro maturasyon: Kanserfobik hastalarda bir çözüm olabilir mi? Bir pilot çalışma

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Abstract

Objective: To investigate whether letrozole priming could be used efficiently in patients undergoing *in vitro* maturation (IVM) as compared with follicle-stimulating hormone (FSH) priming.

Materials and Methods: This is a retrospective analysis of 63 patients who underwent IVM due to the high risk of Ovarian Hyperstimulation syndrome (OHSS) (n=39), cancerophobia (n=16), and desire for IVM after failed in vitro fertilization attempts (n=8). Forty-two patients received FSH priming and 21 patients received letrozole priming.

Results: The patients who had FSH or letrozole priming were statistically similar with respect to age, body mass index, duration of infertility, basal antral follicle count, serum anti-Müllerian hormone levels, and IVM indications (p>0.05 for all). When compared with the FSH priming group, the number of germinal vesicle oocytes, metaphase II and fertilized oocytes were significantly higher (p=0.003, p=0.001, and p=0.016, respectively), but the number of metaphase I oocytes was significantly lower in the letrozole priming group (p=0.002). The patients who received FSH and letrozole priming had statistically similar rates of implantation (33.3% vs 37.0%, p=0.709), clinical pregnancy (31.5% vs 33.3%, p=0.848), twinning (1.9% vs 3.7%, p=0.611), and live birth (24.1% vs 29.6%, p=0.682).

Conclusion: Potential indications for IVM include patients with increased risk for OHSS and contraindication for hyperestrogenism. Aromatase inhibitors can be used to preserve the fertility of patients with estrogen-sensitive cancers. Letrozole priming appears to be an efficient approach in patients who undergo IVM, with likely less cost than FSH priming.

Keywords: Cancerphobia, *in vitro* maturation techniques, letrozole, oocytes, Ovarian Hyperstimulation syndrome, pregnancy

Öz

Amaç: Bu çalışma, *in vitro* maturasyona (IVM) giren hastalarda letrozol kullanımının etkinliğinin folikül uyarıcı hormon (FSH) ile öncüllenmiş uygulama ile karşılaştırılmasını araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Bu retrospektif çalışma, yüksek Yumurtalık Hiperstimülasyon sendromu (OHSS) riski (n=39), kanserfobisi (n=16) ve başarısız tüp bebek tedavisi sonrası IVM denemk isteyen (n=16) IVM'ye giren 63 hastanın analizidir. Kırk iki hasta FSH ile öncüllenmiş, 21 hasta ise letrozol ile öncüllenmiştir.

Bulgular: FSH veya letrozol öncüllenmiş olan hastalar yaş, vücut kitle indeksi, infertilite süresi, bazal antral folikül sayısı, serum anti-Müllerian hormon seviyeleri ve IVM endikasyonları açısından istatistiksel olarak benzerdi (tümü için p>0,05). FSH öncüllenmiş grupla karşılaştırıldığında, letrozol öncüllenmiş grupta germinal vezikül oositleri, Metafaz II ve döllenmiş oositlerin sayısı anlamlı olarak daha yüksekti (sırasıyla p=0,003, p=0,001 ve p=0,016); ancak Metafaz I oositlerinin sayısı letrozol öncüllenmiş grubunda anlamlı olarak daha düşüktü (p=0,002). FSH ve letrozol öncüllenmiş hastalarda istatistiksel

PRECIS: Letrozole priming *in vitro* maturation protocol has promising results in cases with cancerphobia and patients with history of OHSS. The outcomes are comparable with FSH priming IVM.

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olarak benzer implantasyon oranları (%33,3'e karşı %37,0, p=0,709), klinik gebelik (%31,5'e karşı %33,3, p=0,848), ikiz gebelik (%1,9'a karşı %3,7, p=0,611) ve canlı doğum (%24,1'e karşı %29,6, p=0,682) gözlenmiştir.
Sonuç: IVM için potansiyel endikasyonlar; artmış OHSS riski ve hiperinsulinizm için kontrendikasyon olmasıdır. Aromataz inhibitörleri östrojen sensitif kanserlerde fertilite koruma amaçlı kullanılabilmektedir. Letrozol ile öncülleme IVM tedavisine giren hastalarda etkin bir yaklaşım olarak görülmekte ve FSH öncüllenmiş tedaviden olasılıkla daha uygun maliyette olmaktadır.
Anahtar Kelimeler: Kanserfobi, *in vitro* maturasyon teknikleri, letrozol, oositler, Ovarian Hiperstimulasyon sendromu, gebelik

Introduction

In vitro maturation (IVM) refers to the retrieval of immature oocytes from antral follicles, with the final stages of meiosis completed during *in vitro* culture[1]. Potential indications for IVM include patients with increased risk for Ovarian Hyperstimulation syndrome (OHSS), patients with limited time for ovarian stimulation, and patients with a contraindication for elevated concentrations of estradiol[1-3].

The primary benefits of IVM are the reduction in gonadotropin exposure, the subsequent decrease in the risk of OHSS, and facilitation of oocyte retrieval in oncology patients who need to undergo gonadotoxic treatment without adequate time for ovarian stimulation[3,4]. Another benefit of IVM is the avoidance from ovarian stimulation-related hyperestrogenism in oncology patients with hormone-sensitive tumors[4,5].

Letrozole is an aromatase inhibitor that blocks the conversion of androgens into estrogens in the ovarian milieu[6]. Letrozole exerts its primary action by increasing endogenous secretion of follicle-stimulating hormone (FSH) and a secondary action by giving rise to a hyperandrogenic microenvironment, which triggers the development of primordial follicles[7]. Therefore, it has been hypothesized that letrozole could be used to achieve ovarian stimulation in women with hormone-sensitive tumors without exposing them to sustained elevated estrogen levels[8]. The avoidance from hyperestrogenism might also help to relieve the anxiety of infertile women who might be concerned about the long-term probability of developing estrogen-sensitive cancers while they are undergoing an assisted reproductive cycle[8-10]. On the other hand, the emergence of a hyperandrogenic microenvironment might be beneficial in cases of oocyte maturation arrest[11,12].

This study aimed to investigate whether letrozole priming could be used efficiently for patients who were to undergo IVM treatment due to a high risk of OHSS, desire for IVM or fear for estrogen-sensitive cancers.

Materials and Methods

This is a retrospective analysis of 63 patients who underwent IVM treatment due to the high risk of OHSS (n=39), fear for estrogen-sensitive cancers (n=16), and desire IVM after IVF failure (n=8) at Samsun Medicana International Hospital between September 2017 and January 2020. The primary risk factors for OHSS included young age (<33 years), polycystic ovaries on transvaginal ultrasonography (>24 antral follicles), and previous OHSS[13]. The women with cancerophobia were those with Polycystic Ovary syndrome (PCOS) who had fibrocystic breast disease (n=10), a family history of breast cancer (n=3), and family history of endometrium cancer (n=3). This study was approved by the Institutional Review Board of the study center (approval no: 3/2020) and conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

Forty-two patients received FSH- human chorionic gonadotropin (hCG) priming and 21 patients received letrozole-hCG priming. Cancerophobia in the letrozole priming group was related to fibrocystic breast disease (n=5), positive family history of breast cancer (n=1) or endometrial cancer (n=1). Patients with hyperprolactinemia, Cushing's syndrome, non-classic congenital adrenal hyperplasia, adrenal and ovarian androgen-secreting tumors were excluded. Women whose partners had azoospermia, cryptozoospermia, and severe oligoasthenoteratozoospermia were also excluded.

Gonadotropin and Letrozole Priming

The standard protocol for IVM indicated FSH-hCG priming at the study center, as previously described[14]. IVM with letrozole priming was used only for the aforementioned indications. Beginning on the 3rd day of a spontaneous or an induced menstrual cycle, 21 patients had a daily dose of 5 mg letrozole (Femara®, Novartis, Basel, Switzerland) for 3 days, and 42 patients received recombinant FSH (Gonal-f®, Merck-Serono, Geneva, Switzerland) at a daily dosage of 75 IU for 3 days. Either 10,000 IU of urinary hCG (Pregnyl®, Organon, Amsterdam, Netherlands) or 250 µg recombinant hCG (Ovitrelle®, Serono, Geneva, Switzerland) was administered when the leading follicle size was 10 to 12 mm and endometrial thickness was at least 8 mm on transvaginal ultrasonography in the mid-sagittal plane. Under the guidance of transvaginal ultrasonography, oocyte pickup was performed using a 17-gauge double-lumen aspiration needle with an aspiration pressure of 80 mmHg 36-38 hours after hCG administration.

In Vitro Maturation

Follicular aspirates were collected into 14 mL Falcon tubes (Falcon, Code 352001), which were kept at 37 °C and filtered using a strainer (Falcon-70 µm pore size-, Code 352350). Under a stereomicroscope (Olympus SZ 61, Shibuya, Tokyo, Japan), oocytes were isolated into the perimeter of a Falcon center-well dish containing flushing medium without heparin and subsequently incubated for two to three hours in IVM media.

Upon completion of oocyte collection, the final medium was prepared using commercially available IVM medium (Vial 2 Medi-Cult IVM System), 0.5 mL of patient serum, 50 µL FSH prepared from a stock solution of 0.075 IU/mL (75 IU GONAL-f was diluted with 10 mL IVM medium and 5 µL hCG,

and then incubated at 37 °C and 6% CO_2 and 5% O_2. After incubation in LAG medium, oocytes were placed equally in the wells of a four-well dish (Nunc, Roskilde, Denmark) containing 0.6 mL of final IVM medium covered with liquid paraffin and incubated for 26-28 hours. Nuclear maturity of oocytes was not assessed any further.

Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection (ICSI) was performed in all IVM cycles. The spermatozoa were prepared using a three-layer PureSperm gradient (Codes PSB 100 and PS100-100, Mölndal, Gothenburg, Sweden). Removal of the cumulus and corona cells was performed in a hyaluronidase-containing medium using Pasteur pipettes after a 26-28 h incubation period. The oocytes were then transferred to U-IVF medium for culture. All ICSI procedures were performed in Falcon Petri dish with droplets of PVP containing medium for sperms (Vitrolife, Code 10111, 5x0.1 mL) and droplets of flushing medium without heparin for oocytes.

When the ICSI procedure was completed, the oocytes were placed into ISM 1 medium for culture. After the oocyte fertilization was evaluated in droplets of ISM 1 medium, incubation of fertilized oocytes was continued for 24-hours (which made up 48-hours in total). The first fertilization check, which occurred 16-18 h after ICSI was determined by the presence of two distinct pronuclei and two polar bodies within the oocytes in ISM 1 medium through an inverted microscope at x200 magnification. This was repeated 24 hours later.

The classification system introduced by Veeck was used for the evaluation of embryo grade on the third day of culture. The embryos were graded as follows: Grade 1 - embryo with blastomeres of equal size, no cytoplasmic fragments; Grade 2 - embryo with blastomeres of equal size, minor cytoplasmic fragments or blebs: Grade 3 - embryo with blastomeres of distinctly unequal size, none or few cytoplasmic fragments[15].

Embryo transfer was performed using a Wallace embryo transfer catheter (Wallace, UK) under abdominal ultrasonography guidance and placed 1.5 to 2.5 cm from the fundus. Women aged younger than 35 years could only have a single embryo transferred, and a maximum of two embryos could be transferred to women aged over 35 years. Taking endometrial thickness into consideration, fresh embryo transfers were performed on day 3 or day 5. To prepare the endometrium for embryo transfer, an oral estrogen tablet was administered at a daily dosage of 4 mg (Estrofem®, Novo Nordisk, Bagsværd, Denmark) beginning from the third day of menstrual cycle. Luteal phase support was begun the day following OPU, which was provided with a vaginal progesterone capsule at a daily dosage of 200 mg (Progestan®, Koçak Farma, İstanbul, Turkey). Estrogen tablets and progesterone capsules were continued until the first fetal heartbeat was detected.

Clinical pregnancy was defined as the presence of at least one gestational sac in the uterus. No IVM cycles were cancelled in this study.

Statistical Analysis

Collected data were analyzed using the Statistical Package for the Social Sciences version 22.0 software (SPSS IBM Inc., Armonk, NY, USA). Continuous variables are expressed as mean ± standard deviation and categorical variables are denoted as numbers or percentages. Continuous variables were compared using Student's t-test or the Mann-Whitney U test, whereas categorical variables were compared using the chi-square test. Two-tailed p values ≤0.05 were regarded as statistically significant. A post-hoc analysis was performed to determine that a cohort size of 63 women (undergoing IVM for minimizing OHSS risk and preventing oocytes maturation arrest) had 67.6% power to detect a difference at the 0.05 significance level.

Results

Table 1 compares the baseline characteristics of the 21 patients who had letrozole priming and 42 patients who received FSH priming for IVM treatment. There were no significant group differences in age, body mass index, duration of infertility, basal antral follicle count, and serum AMH concentrations (p>0.05 for all). The patients who underwent FSH and letrozole priming were also statistically similar in the aspect of IVM indications (Table 2). Table 3 summarizes the laboratory findings of the patients.

Table 1. Baseline characteristics of the patients

	FSH priming	Letrozole priming	p
Age (years)	29.2±4.8	31.1±4.2	0.129
Body mass index (kg/m^2)	27.27±6.02	25.16±4.35	0.159
Duration of infertility (years)	6±3.19	6.1±3.4	0.87
Antral follicle count (n)	15.7±2.1	7.7±6.6	0.198
Anti-Müllerian hormone (ng/mL)	5.56±1.35	6.04±1.36	0.191

Values are given as mean ± SD; FSH: Follicle-stimulating hormone, SD: Standard deviation

Table 2. Indications for *in vitro* maturation

	FSH priming	Letrozole priming	p
Oocyte maturation arrest	4 (9.5)	4 (19)	0.285
PCOS patients with cancerophobia	9 (21.4)	7 (33.3)	0.306
Increased risk for OHSS	29 (69.1)	10 (47.7)	0.099
Young age (<33 years)	3 (7.1)	-	0.209
Polycystic ovaries on TV-USG	15 (35.7)	5 (23.8)	0.338
Previous OHSS	11 (26.2)	5 (23.8)	0.838

Values are given as number (percentage); FSH: Follicle-stimulating hormone, PCOS: Polycystic Ovary syndrome, OHSS: Ovarian Hyperstimulation syndrome; TV-USG: Transvaginal ultrasonography

Both the FSH and letrozole priming groups were statistically similar concerning the endometrium thickness on the hCG day, time to oocyte pick up, retrieved oocytes counts, and single or double embryo transfers (p>0.05 for all). When compared with the FSH priming group, a significantly higher number of germinal vesicle oocytes, metaphase II, and fertilized oocytes (p=0.003, p=0.001, p=0.016, respectively) and a significantly lower number of metaphase I oocytes and grade 3 embyros were detected in the letrozole priming group (p=0.002, p=0.007, respectively). The number of three-cell embryos was also significantly lower in the letrozole priming group when compared with the FSH priming group (p=0.007).

Table 4 shows the pregnancy outcomes of the patients. The patients who received FSH and letrozole priming had statistically similar rates of implantation (33.3% vs 37%, p=0.709), clinical pregnancy (31.5% vs 33.3%, p=0.848), twin pregnancy (1.9% vs 3.7%, p=0.611), and live birth (24.1% vs 29.6%, p=0.682).

Discussion

By definition, IVM refers to the maturation of collected immature oocytes under the influence of hormones that exist within the culture media. Thus, hormones are not administered to patients undergoing IVF treatment[16,17]. However, the general opinion about IVM is that a short priming protocol with FSH stimulation increases the chance for oocyte maturation and implantation in patients with PCOS. This short priming protocol involves the administration of FSH at a dose of 75 IU to 150 IU for a period of two to five days. FSH stimulation at the beginning of a cycle increases the number of immature oocytes retrieved and thus clinical pregnancy rates in IVM cycles[18-20]. Accordingly, this study adopted an IVM protocol indicating the administration of recombinant FSH at a dose of 75 IU for three days.

In this study, 16 patients with PCOS refused to receive FSH priming because they had cancerophobia due to fibrocystic breast disease and a positive family history for breast and endometrium cancer. Therefore, priming was performed using an aromatase inhibitor, namely letrozole, in seven patients with PCOS.

In this study, the primary indications for IVM treatment were the avoidance from OHSS and previously failed IVF desiring IVM. It is well known that IVM is the only assisted reproduction technique that has been proved to be devoid of OHSS risk. Recent meta-analyses reported that patients treated with IVM had significantly higher implantation and clinical pregnancy rates, as well as significantly lower miscarriage and cycle cancellation rates[22,23]. The maturation of human oocytes is naturally arrested at the germinal vesicle stage when the oocytes need gonadotropin stimulation and the metaphase II stage when oocytes are waiting for fertilization. A therapeutic approach for failed IVF could be the IVM of immature oocytes within culture media enriched with factors necessary for oocyte maturation[11,12], if compromised ***in vivo***.

Due to the wide variations in priming protocols, varying numbers between 8% and 40% have been specified as the clinical pregnancy rates and live birth rates in IVM cycles[24]. Similarly, our previous study reported clinical pregnancy rates of 44.6% and 44.7% and live birth rates of 34.9% and 34.2% for single-embryo transfer and double-embryo transfers in IVM cycles with FSH priming[14]. As for the present study, the clinical pregnancy and live birth rates were respectively 31.5%

Table 3. Laboratory findings of the patients

	FSH priming	Letrozole priming	p
Endometrial thickness at hCG day (mm)	9.07±1.25	9.63±1.68	0.138
Time to oocytes pick up (days)	13.2±1.8	14.0±1.8	0.123
Retrieved oocytes (n)	12.8±6.8	14.6±3.6	0.273
Germinal vesicle oocytes (n)	3.4±1.0	8.1±6.5	0.003*
Metaphase I oocytes (n)	3.8±1.5	2.6±0.7	0.002*
Metaphase II oocytes (n)	6.3±3.1	9.4±2.7	0.001*
Fertilized oocytes (n)	5.5±2.8	6.8±1.5	0.016*
Embryo transfer (n, %)			0.621
Single embryo	30 (71.4)	15 (71.4)	-
Double embryo	12 (28.6)	6 (28.6)	-
Embryo grade (n, %)			0.007*
Grade 1	5 (2.4)	7 (23.8)	-
Grade 2	28 (57.1)	15 (61.9)	-
Grade 3	21 (40.5)	5 (14.3)	-
Embryo quality (n, %)			0.007*
One-cell embryo	5 (4.8)	5 (18.5)	-
Two-cell embryos	4 (2.4)	7 (26)	-
Three-cell embryos	31 (66.7)	10 (37)	-
Four-cell embryos	14 (26.1)	5 (18.5)	-

Values are given as mean ± SD or number (percentage) as indicated; FSH: Follicle-stimulating hormone, hCG: Human chorionic gonadotropin, *significant difference, SD: Standard deviation

Table 4. Comparison of pregnancy outcomes of two groups

	FSH priming	Letrozole priming	p
Implantation rate per transfer	18/54 (33.3)	10/27 (37)	0.709
Clinical pregnancy rate per transfer	17/54 (31.5)	9/27 (33.3)	0.848
Singleton pregnancy rate per transfer	16/54 (29.6)	7/27 (25.9)	0.688
Twin pregnancy rate per transfer	1/54 (1.9)	1/27 (3.7)	0.611
Live birth rate per transfer	13/54 (24.1)	8/27 (29.6)	0.682

Values are given as number (percentage), FSH: Follicle-stimulating hormone

and 24.1% in IVM cycles with gonadotropin priming. Although clinical pregnancy and live birth rates tended to be higher in IVM cycles with letrozole priming (33.3% and 29.6%), there were no statistically significant differences.

A new indication for IVM has been defined as a convenient therapeutic approach for infertile patients who have been diagnosed as having malignancies and scheduled for oncofertility treatment. The basic rationale for this definition is the feasibility of IVM for preserving oocytes and embryos and thus, future fertility, whenever conventional *in vitro* fertilization is not an option. A potential factor that might delay the oncology treatment can also be avoided because IVM prevents the risk of OHSS. Additionally, this technique can be used to replace an IVF cycle that would otherwise end up with excessive follicular growth and subsequent cycle cancellation[25,26].

Another advantage of IVM treatment is that it can be started immediately at any time of the menstrual cycle without stimulating ovaries. Therefore, IVM emerges as an appropriate technique for preserving the fertility of oncology patients in whom the initiation of chemotherapy, radiotherapy or surgical treatment cannot be delayed[1,2]. Another advantage of this assisted reproduction technique is that serum estrogen concentrations remain low throughout an IVM cycle. Therefore, IVM has been established as a safe and effective method for fertility preservation in patients with estrogen-sensitive tumors[25,26].

It is also well known that aromatase inhibitors can be used as single agents or adjuncts to FSH-containing ovulation induction regimens for preserving the fertility of patients with estrogen-sensitive breast cancer. Aromatase inhibitors would reduce supra-physiologically serum concentrations of estradiol, suppress local production of estrogen within the tumor tissue, and induce follicular growth in women with estrogen-sensitive malignancies. Combining gonadotropins with aromatase inhibitors would augment the ovarian stimulation without a profound increase in serum estradiol levels[27,28].

Conclusion

The findings of this study suggest that IVM treatment with letrozole priming might be an efficient approach for patients who have a high risk for OHSS or fear for estrogen-sensitive tumors. However, these findings should be interpreted carefully as their power is limited by its retrospective design, small cohort size, technical inadequacy for cryopreservation, and lack of long-term data. Further research is warranted to clarify the clinical implications of letrozole priming in IVM cycles. A prospective study of letrozole-primed IVM should be considered.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Review Board of the study center (approval no: 3/2020).

Informed Consent: All participants gave written informed consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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A randomized pilot study of electro-acupuncture treatment for hysterosalpingography pain relief and related anxiety

Histerosalpingografi ilişkili ağrı ve anksiyetede elektro-akupunktur tedavisi: Randomize pilot çalışma

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Abstract

Objective: To investigate the effect of acupuncture on reducing pain and anxiety related to hysterosalpingography (HSG).

Materials and Methods: A total of 107 patients planned to undergo HSG were randomly separated into 3 groups; the acupuncture group (n=36), intramuscular diclofenac sodium group (n=35), and control group (n=37). In the acupuncture group, electro-acupuncture was applied to specified points for 20 mins before the procedure. In the intramuscular diclofenac sodium group, an intramuscular injection of 75 mg diclofenac sodium was applied 30 mins before the procedure. No analgesics were administered to the patients in the control group before intervention. Pain was evaluated with a Visual Analog scale (VAS) and anxiety with the State-trait Anxiety inventory -state (STAI-S), preoperatively and at specified times postoperatively.

Results: The VAS scores at 1 and 5 minutes after HSG were similar in acupuncture group and intramuscular diclofenac sodium group, and significantly lower than in the control group. At 30 mins postoperatively, there was no significant difference between the 3 groups in respect of the VAS scores. The STAI-S scores at 1 hour preoperatively were similar in all the groups (p=0.563). In the comparisons of the STAI-S values at preoperative 5 mins, following acupuncture in acupuncture group and the diclofenac injection in intramuscular diclofenac sodium group, and at postoperative 30 mins, the acupuncture group values were determined to be statistically significantly lower than those of the other groups (p<0.001, p<0.001).

Conclusion: Acupuncture has similar effects on the reduction of pain as other analgesics and reduces anxiety. It can therefore be used in HSG in suitable clinics.

Keywords: Acupuncture, anxiety, hysterosalpingography, pain relief

Öz

Amaç: Akupunkturun histerosalpingografiye (HSG) bağlı ağrı ve anksiyeteyi azaltıcı etkisini araştırmak.

Gereç ve Yöntemler: HSG çekimi planlanan 107 hasta randomize olarak üç gruba ayrıldı. Akupunktur grubu (n=36), İntramüsküler diklofenac sodyum grubu (n=35), kontrol grubu (n=37). Akupunktur grubuna işlem öncesi, belirlenen noktalara 20 dakika elektroakupunktur uygulandı. İntramüsküler diklofenac sodyum grubunda hastalara işlemden 30 dakika önce 75 mg diklofenac sodyum intramüsküler uygulandı. İşlem öncesi ve sonrası belirlenen zamanlarda Görsel Anolog ölçeği (VAS) ağrı ve State-trait Anxiety inventory-state (STAI-S) ile anksiyete ölçümleri yapıldı.

Bulgular: HSG'den sonra 1. ve 5. dakika VAS skorları akupunktur ve intramüsküler diklofenac sodyum gruplarında birbirlerine benzer ve kontrol grubuna göre anlamlı düzeyde düşük bulunurken 30. dakikadaki VAS skorları arasında üç grup içerisinde anlamlı bir fark yoktu. STAI-S skorları işlemden 1 saat önce tüm gruplarda benzer iken (p=0,563). İşlem öncesi 5. dakikada yani akupunktur grubunda akupunktur sonrası, intramüsküler diklofenac sodyum

PRECIS: Acupuncture has similar effects on the reduction of pain as other analgesics and reduces anxiety.

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grubunda ise diclofenac enjeksiyonu sonrası ve işlem sonrası 30. dakikada saptanan STAI-S değerleri karşılaştırıldığında akupunktur grubunda diğerlerine göre daha düşük seviyelerde izlenmiştir (p<0,001 ve p<0,001).
Sonuç: Akupunkturun ağrıyı azaltıcı etkisi diğer analjeziklerle benzer olması ve ilave olarak anksiyete azaltıcı etkinliği nedeniyle uygun kliniklerde HSG çekimlerinde kullanılabilir.
Anahtar Kelimeler: Akupunktur, anksiyete, histerosalpingografi, ağrı kesici

Introduction

Infertility affects approximately 15% of the general population worldwide and 40-50% is attributed to female infertility. It is caused by many factors, and fallopian tube abnormalities cause about 30-35% of cases of female infertility[1]. Hysterosalpingography (HSG) is a relaible and cost-effective method used in the determination of both tubal pathologies and tubal patency, and uterine and peritoneal pathologies in the investigations into the etiology of infertility[2]. Although HSG is a procedure that does not require cervical dilatation or general anaesthesia, the procedure can be painful. Up to 72% of patients complain of pain from the procedure[3].

The mechanisms causing pain may include cervical instrumentation, and irritation of contrast medium in uterine cavity distension, and peritoneal irritation of contrast medium that has spread to the abdomen. In addition, taking the uterus into traction by holding the cervix with a tenaculum increases local prostaglandin synthesis and can lead to uterine contractions and pain[4]. Patients think that this procedure will be painful and are reluctant to have the test, which also causes anxiety. Many different randomised controlled studies have investigated many different agents and different application methods to reduce pain during HSG[5-8], and there are reviews in literature that have evaluated these studies[9-11]. However, the majority of these studies have limitations such as heterogenity in the randomisation, lack of blinding, patient selection criteria, differences in drug doses and in the pain score measurements. Therefore, no optimal method has been found as yet, which can be recommended to alleviate pain during the HSG procedure.

Complementary treatments have started to be more widely used in modern medicine, and of these, acupuncture applications have shown efficacy, especially in acute pain syndromes[12] and chronic pain[13]. Kiran et al.[14] determined that acupuncture and non-steroid anti-inflammatory drugs (NSAIDs) have similar effects in patients with primary dysmenorrhea. In recent randomised controlled studies, Smith et al.[15] reported that acupuncture decreased anxiety and increased quality of life in patients applied with *in vitro* fertilization.

Therefore, the aim of this study was to investigate, for the first time in literature, the effect of acupuncture in reducing pain and anxiety in patients undergoing HSG.

Materials and Methods

Approval for the study was granted by the Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University (decision no: 08, session: 2015/05). Patients who admitted to our Assisted Reproductive Techniques clinic with the desire to have a child and planned to undergo HSG were included in our study. Informed consent was obtained from all the patients. Age, infertility duration, gravida and smoking status of all patients participating in the study were registered. At the beginning of the study, a pilot study was conducted with 10 patients from each group and the minimum number of patients in each group with α=0.05 and 90% power was found to be 27 in the power analysis. There with, 133 patients were planned to be included in the study at first. However, the study was conducted with the remaining 107 patients after excluding the ones allergic to radiopaque substance (n=1) and NSAID (n=3), those with cervical-endometrial infection (n=5), those with previously defined NSAID drug-related side effects (n=5), those with cervical surgical anamnesis (n=1), those who were found to have acute pelvic inflammatory disease (n=3) and those who did not want to participate in the study. The remainig 107 subjects were divided into three groups after computer-generated randomization; the acupuncture (ACU) group (n=36), intramuscular diclofenac sodium (IMDS) group (n=35), and the control group (n=37).

For the patients in the ACU group, taking anxiety into consideration, the points selected and identified to decrease the pain and stress that can develop during the taking of HSG were those which are most widely used in the treatment of dysmenorrhea and anxiety. All the ACU procedures were applied by the same physician (A.D.) who has experience on ACU procedures for more than ten years. The H7, Du-20, Liv-3, P-6, HT-7, PC-6, LI 4, LI 10, SP-6, LR-3, ST-36, GB-26, CP-15, ST-28 and Ren-4 points were stimulated using sterile, disposable, steel needles with a single-sided point and length and diameter of 0.30x40 mm and 0.25x25 mm, placed bilaterally in a single session at a depth of 10-50 mm and angled at 45-90° to the skin. ACU was applied to these points for 20 minutes using Acutens SMS-205, 5-channel, 1-200 Hz, 1-10 mA, at 1-20 Hz frequency and current at maximum 10 mA intensity[16]. The HSG procedure was applied within 3 hours of the ACU application.

For the patients in the IMDS group, 75 mg diclofenac sodium (Diclomec®, Abdi İbrahim Medicin, Turkey) was administered intramuscularly one hour before the HSG procedure[17].

No medication or procedure was applied to the control group patients before HSG.

The randomisation of patients to the 3 groups was performed by a single physician (M.G.) blinded to the patients. HSG was applied in the Outpatients clinic at 1-3 days after the end of menstruation, in the follicular phase of the menstrual cycle. HSG was performed under fluoroscopy guidance with the patient in the lithotomy position. A sterile speculum was placed in the

vagina, and following observation of the cervix, local cleaning was applied with 3% povidone-iodine. Then, depending on the cervix position, the anterior or posterior cervical lip was held with a tenaculum, and a Rubin HSG cannula was gently placed in the cervical canal. The speculum was removed and 10-20 mL water-soluble, radio-opaque solution (Urografin® 76% 100 mL, Bayer AG, Turkey) was used as a contrast medium and injected slowly under spot fluoroscopy. After completion of the procedure, all the instruments were removed and the patient was transferred to a bed.

A Visual Analog scale (VAS) was used in the evaluation of pain severity, and the State-trait Anxiety inventory- state (STAI-S) in the evaluation of patient anxiety. All the VAS and STAI-S scoring was performed by a single physician (A.U.) blinded to the groups. In the VAS system, the patient is instructed to place a mark on a 100 mm horizontal line corresponding to the level of pain felt, where 0 =no pain and 10 =intolerable pain. The questions on the STAI-S form were asked directly to the patients and the results were recorded. Both evaluations were applied at 1 min and 5 min before the HSG procedure and at 30 minutes after the procedure and all the results were recorded. In the evaluation of anxiety, the changes over the specified time period were evaluated.

Statistical Analysis

Data obtained in the study were analysed statistically using SPSS 22.0 vn 22 software (IBM Statistics for Windows version 22, IBM Corporation, Armonk, NY, USA) and PAST 3 software (Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. Paleontological statistics). To assess the conformity of data to normal distribution, the Shapiro-Wilk test was applied to data with single variables and the Mardia test (Dornikand Hansen omnibus) to data with multiple variables. The Levene test was used to evaluate variance homogeneity. Parametric methods were used in the analyses of variables with homogenous variance and normal distribution and non-parametric methods were used for variables not showing homogenous variance and normal distribution. In the comparisons of independent multiple groups with each other, the One-Way ANOVA (Robust test: Brown-Forsythe) and Kruskal-Wallis tests were used, and for the post hoc analyses, the non-parametric post hoc test (Miller, 1966). To examine the interaction of repeated measurements of dependent variables according to the groups, the General Linear Model Repeated Anova (Wilks Lambda) was used and the LSD test for post hoc analysis. Quantitative data were presented in the tables as mean ± standard deviation and median range (IQR or minimum-maximum) values, and categorical data as number (n) and percentage (%). The data were examined at a 95% confidence interval. A value of $p<0.05$ was accepted as statistically significant.

Results

As shown in Table 1, there was no statistical difference found among the mean age, infertility duration, gravida and smoking status of the patients participating in the study (all $p>0.05$). When the VAS scores 1 and 5 minutes after the procedure were examined, it was found that the values in the control group were statistically significantly higher than the ACU and IMDS groups, and there was no difference between the ACU and IMDS groups ($p<0.001$ and $p=0.002$, respectively). There was no statistical difference found between the VAS scores at the 30th minute after the procedure ($p=0.625$) (Table 2, Figure 1). There was no statistically significant difference found between the STAI-S values determined at the first hour of the procedure, that is, just before the application of ACU and intramuscular Diclofenac ($p=0.563$). When STAI-S values determined at the 5th minute before the procedure, that is, after ACU in the ACU group, and after Diclofenac injection in the IMDS group, were examined the values in the ACU group were found to be statistically significantly lower than the IMDS and control groups while that difference was not determined between the IMDS and control groups ($p<0.001$) (Figure 2). STAI-S scores 5 minutes before the procedure decreased only in the ACU group compared to the STAI-S score 1 hour before the procedure, but

Table 1. Demographic characteristics of the cases

	Acupuncture group (n=36)	Intramuscular diclofenac group (n=35)	Control group (n=37)	p
Age (years)	26.43 (21-35)	25.93 (20-36)	26.31 (21-35)	0.789
Duration of infertility (years)	2.65±0.86	2.89±0.85	3.1±1.1	0.281
Gravida	0.25±0.56	0.26±0.56	0.42±0.63	0.697
Smoking status (%)	7/36 (19.4%)	8/35 (22.8%)	7/37 (18.9%)	0.724

Data are expressed as median Range, (maximum-minimum), mean ± standart deviation or n (%)

Table 2. VAS scores at 1, 5, and 30 mins after HSG

	Acupuncture group (n=36)	Intramuscular diclofenac group (n=35)	Control group (n=37)	p
VAS (1 minute after HSG)	6.0±1.7	6.4±1.2	7.3±0.9[ab]	<0.001
VAS (5 minutes after HSG)	3.2±1.3	3.5±1.0	4.1±0.9[ab]	0.002
VAS (30 minutes after HSG)	1.0±1.3	1.1±0.8	1.2±0.7	0.625

General linear model repeated ANOVA (Wilks' Lambda), One-Way ANOVA (Brown-Forsythe), Post-hoc test: LSD-Games Howell, Mean ± standard deviation, VAS: Visual Analog scale, HSG: Hysterosalpingography
[a]statistically significant compared to the intramuscular diclofenac group
[b]statistically significant compared to the acupuncture group

increased in the IMDS and control groups. When the STAI-S scores 30 minutes after the HSG procedure were examined, the values in the ACU group were found to be statistically significantly lower than the IMDS and control group, similar to the 5th minute before the procedure, but this difference was not determined between the IMDS and control groups (p<0.001) (Table 3). When the changes in the STAI-S scores (between 1 hour before-5 minutes ago, 1 hour before-30 minutes later and 5 minutes before-30 minutes later, respectively) were analyzed, the changes in STAI-S scores in the ACU group were found to be statistically significant for all three change values

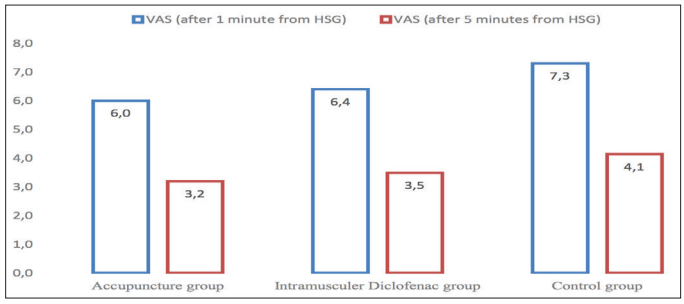

Figure 1. VAS scores 1 minute before and 5 minutes after HSG
VAS: Visual Analog scale, HSG: Hysterosalpingography

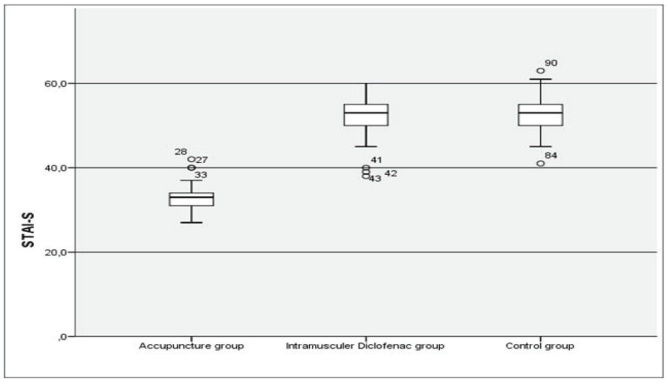

Figure 2. STAI-S scores 5 minutes after HSG
HSG: Hysterosalpingography, STAI-S: State-trait Anxiety inventory -state

than the IMDS and control group while this difference was not determined between the IMDS and control groups (p<0.001, p<0.001 ve p<0.001, respectively) (Table 3).

Discussion

The results of the study showed that ACU reduced the pain scores before and after the HSG procedure as effectively as the NSAID diclofenac. It was also determined that unlike diclofenac, ACU had the effect of reducing anxiety related to the procedure.

There are many studies on the subject of pain relief in HSG that have investigated the types of pharmacological drugs used and the methods of use. A systematic Cochrane review in 2015 on the subject of pain releief in HSG analysed 23 prospective, randomised studies. Although the level of evidence was low, it was concluded that topical anaesthetics and intravenous opioids could be effective in reducing pain during the procedure, but this effect was not seen after the procedure. It was reported that there was not sufficient evidence that other analgesic methods could be effective[10]. In a review by Ahmad et al.[9] on the subject of pain-relief in office-setting gynaecological interventions, there was reported to be no benefit of oral NSAIDs within the first 30 mins and 30 mins after the procedure, but when local anaesthetics were used, it was shown that even if not in the first 30 mins, there could be beneficial effects after 30 mins.

The majority of studies in literature related to reducing pain with non-pharmacological methods are studies where catheters have been used. It has been suggested that catheters used during HSG could have different effects on pain. In studies by Austin et al.[18] and Varpula[19], it was determined that the use of flexible balloon catheters instead of a traditional metal cannula was not effective in reducing pain, while De Mello et al.[20] reported in a study published in 2006 that the use of flexible balloon catheters resulted in less pain. Stoop et al.[21] investigated the efficacy of fast-release orodispersible tramadol, as a different analgesic method, in cases where traditional metal cannula and balloon catheter were used, and reported that it was effective independently of the type of catheter used.

Table 3. STAI-S values at 1 hour and 5 minutes before and 30 minutes after HSG and the changes in those intervals

	Acupuncture group (n=36)	Intramuscular diclofenac group (n=35)	Control group (n=37)	p
STAI-S (1 hour before HSG)	43 (5.5)	45 (8)	44 (4)	0.563
STAI-S (5 minutes before HSG)	33 (3)[ab]	53 (5)	53 (5)	<0.001
STAI-S (30 minutes after HSG)	32 (3)[ab]	43 (4)	41 (4)	<0.001
Change in STAI-S (1 hour before -5 mis before)	10 (6)[ab]	-9 (5)	-10 (3)	<0.001
Change in STAI-S (1 hour before-30 mins after)	12 (6)[ab]	3 (8)	1 (5)	<0.001
Change in STAI-S (5 mins before-30 mins after)	1 (2)[ab]	10 (7)	10 (5)	<0.001

Kruskal wallis test (Monte Carlo), Post-hoc test: Non-parametric post-hoc test [Miller (1966) median range (interquartile range)]
[a]Statistically significant compared to the control group, HSG: Hysterosalpingography, STAI-S: State-trait Anxiety inventory -state
[b]Statistically significant compared to the intramuscular diclofenac group

ACU is a complementary medicine application with increasingly widespread use. There have started to be wide areas of use especially in the elimination of pain symptoms. Previous studies have shown that the effect mechanisms of ACU are formed on a biological basis. Cheng and Pomeranz[22] reported that the analgesic efficacy of ACU occurred by increasing endogenous opioids and demonstrated that this effect could be removed with naloxane, which is an opioid antagonist. Using functional magnetic imaging technology, another study showed that the stimulation of ACU points affected the the limbic system and structures in both the cortical and subcortical areas in the brain[23]. In a clinical study, Cho et al.[24] determined activity signals in the cingulate gyrus and thalamic region with pain stimuli, and following ACU, reported a decrease both in the signals and in the pain felt by the patient.

Although there are no studies in literature examining the effect of ACU on anxiety engendered by the application of HSG, there are studies related to the effect of lowering anxiety in general. The hypotheses of some of these studies are explained by biochemical mechanisms and some by physiological parameters. Yuan et al.[25] investigated changes in plasma adrenocorticotropic hormone, corticosteroid and platelet 5-HT levels in response to anxiety treatment. Comparisons were made of ACU, pharmacological treatment and combined treatment groups, and similar results were found in the ACU group and the pharmacological treatment group. That the side-effects seen in the pharmacological treatment group were not seen in the ACU group was emphasised as a positive aspect. The effects of ACU on anxiety were investigated by observing changes in the parameters of oxygen saturation and heart rate by Karst et al.[26] and in heart rate and skin conductivity by Shayestehfar et al.[27]. As ACU reduced heart rate in both groups, it was concluded to be effective on anxiety.

To the best of our knowledge, this is the first study in literature to have investigated ACU on this subject. However, there were some limitations to the study, the first of which is that the different stages of HSG (speculum placement, tenaculum placement, opaque medium administration) were not evaluated with VAS. In addition, the side-effects of NSAIDs and ACU were not examined, and when examining the pain scores, the HSG results were not taken into consideration.

Conclusion

Although the use of ACU is restricted in most clinics because of the need for trained practitioners, equipment and time, as it has a similar effect to other analgesics in reducing pain, and effectively reduces anxiety, it can be used in HSG in suitable clinics.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University (decision no: 08, session: 2015/05).

Informed Consent: Informed consent was obtained from all the patients

Peer-review: Internally peer-reviewed.

Authorship Contributions

Design: Z.B., M.B., A.U., Ö.F.B., H.K., Data Collection or Processing: A.D., A.U., M.K., M.G., Editing: Z.B., M.B., A.Y., H.K., Writing: Z.B., M.B., A.Y., Ö.F.B., M.K.

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Antenatal pentoxifylline therapy to prevent endotoxin-induced fetal injury in the preterm goat model

Preterm gebe keçi modelinde endotoksin ile indüklenen fetal hasarın önlenmesinde antenatal pentoksifilin tedavisi

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Abstract

Objective: Pentoxifylline (PTX) has immunomodulatory properties and is known to reduce sepsis-associated infant mortality. We aimed to evaluate maternal oral and intra-amniotic administration of PTX for the prevention of fetal inflammation and injury in a caprine model.

Materials and Methods: Inflammation-mediated fetal injury was induced with maternal granulocyte-colony stimulating factor and intra-amniotic endotoxin at 0.76 of gestation in date-mated pregnant goats. Eight groups were formed (n=4 each): Control, fetal injury, oral 30 mg/kg/day and 60 mg/kg/day PTX for 15 days + fetal injury, intra-amniotic 400 mg/kg and 800 mg/kg estimated fetal weight single-dose PTX with and without fetal injury. Preterm delivery by hysterotomy was performed at 0.80 of gestation to evaluate the fetal and placental effects. Immunochemistry for various markers including interleukins, caspases, cyclooxygenases, vimentin, myelin basic protein, and surfactant proteins were carried out in the fetal lungs, fetal brain, and placenta. Fetal plasma and amniotic fluid interleukins were also evaluated. Kruskal-Wallis H test and Mann-Whitney U test were used for comparisons.

Results: High-dose (60 mg/kg/day) maternal prophylactic oral treatment attenuated endotoxin-related histological injury and was related to low inflammatory marker expressions comparable to the controls ($p>0.05$ except cyclooxygenase 2). Following maternal oral administration, fetal plasma and amniotic fluid levels of the studied interleukins were also lower than the untreated endotoxin-exposed animals ($p<0.05$ for all comparisons). Intra-amniotic PTX was associated with inconsistent results and increased inflammatory markers in some fetuses.

Conclusion: Oral PTX before preterm birth mitigates intrauterine inflammation with neuroprotective effects in the fetus. PTX can be considered as a candidate drug for fetal brain injury prevention in the preterm period.

Keywords: Animal model, endotoxins, fetal brain injury, neuroprotection, pentoxifylline, preterm birth

Öz

Amaç: Pentoksifilin (PTX), immün düzenleyici özellikleri sahiptir ve sepsise bağlı yenidoğan ölümlerini azalttığı bilinmektedir. Mevcut çalışmada, fetal enflamasyon ve hasarın önlenmesinde maternal oral ve intra-amniyotik PTX kullanımının keçi modelinde araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Aynı günde çiftleştirilen keçilerde maternal granülosit koloni uyarıcı faktör ve intra-amniyotik endotoksin ile enflamasyon aracılı fetal hasar 0,76 gestasyonda tetiklendi. Sekiz grup oluşturuldu (her birinde n=4): Kontrol, fetal hasar, 15 gün boyunca 30 mg/kg/gün ve 60 mg/kg/gün oral PTX + fetal hasar, fetal hasarlı veya hasarsız tek doz intra-amniyotik 400 mg/kg ve 800 mg/kg tahmini fetal ağırlıkta PTX. Histeretomi ile 0,80 gestasyonda fetal ve plasental etkilenmenin değerlendirilmesi amacı ile preterm doğum gerçekleştirildi. Fetal akciğer, beyin ve plasentada interlökinler, kaspazlar, siklo-oksijenazlar, vimentin, miyelin bazik protein ve surfaktan proteinleri gibi çok sayıda immünhistokimyasal belirteç ile fetal plazma ve amniyon sıvısında interlökinler değerlendirildi. Karşılaştırmalarda, Kruskal-Wallis H testi ve Mann-Whitney U testi kullanıldı.

PRECIS: Using an inflammation-mediated fetal injury caprine model, we demonstrated neuroptotective effects of maternal pentoxifylline before preterm delivery.

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Bulgular: Yüksek doz (60 mg/kg/gün) maternal profilaktik oral tedavi, endotoksine bağlı histolojik hasarı azaltırken (siklo-oksijenaz 2 dışındaki) enflamatuvar belirteçlerde kontrol seviyelerine benzer (p>0,05) düzelmeler sağladı. Maternal oral uygulama sonrasında, fetal plazma ve amniyon sıvısındaki pro-enflamatuvar interlökinler de sadece endotoksine maruz kalanlardakinden daha düşüktü (tüm karşılaştırmalar için p<0,05). Amniyon içine verilen PTX, bazı fetüslerde enflamatuvar belirteçlerde artışlar ve değişken bir etki profili gösterdi.
Sonuç: Pretem doğum öncesinde oral PTX, intrauterin enflamasyonu azaltarak fetüste nörokoruyucu etki göstermektedir. PTX, preterm dönemde fetal beyin hasarını önleyebilecek bir ilaç adayı olabilir.
Anahtar Kelimeler: Hayvan modeli, endotoksinler, fetal beyin hasarı, nörokorunma, pentoksifilin, preterm doğum

Introduction

Preterm birth is the most important cause of perinatal morbidity and mortality. There is growing evidence that subclinical intra-amniotic (IA) microbial colonization and inflammation is responsible for a significant number of preterm deliveries. Up to 40% of pregnancies that resulted in preterm deliveries were found to have positive inflammatory markers in the second-trimester amniotic fluid[1]. Moreover, increased subclinical IA inflammation is suggested to affect the fetus, with the inflammation probably commencing from the lungs and then propagating to the vulnerable fetal brain. This condition has been called as Fetal Inflammatory Response syndrome (FIRS) and is an indirect consequence of IA microbial colonization, most likely in an ascending manner through the cervix[2,3]. Ultimately, the outcome will be fetal brain injury, which may manifest itself as cerebral palsy (CP) in the long term[4]. Although peripartum hypoxia has conventionally been accused for the development of neonatal encephalopathy, data controlling for confounders show that most cases are indeed associated with antenatal insults to the developing fetal brain[5].

Given the medical and social negative consequences of fetal brain injury, effective antenatal treatments are needed. Magnesium sulfate has been shown to have neuroprotective effects[6], and many clinicians now routinely administer magnesium sulfate treatment before imminent early preterm delivery. However, some recent data have also indicated that magnesium sulfate may not be neuroprotective in the setting of chorioamnionitis[7]. Hence, targeted therapies that have a potential to specifically inhibit initial steps of the inflammatory cascades in the amniotic fluid and the chorioamniotic membranes may be required to decrease fetal brain injury. Moreover, use of fetal neuroprotective agents in a combined form in relatively low cumulative doses may demonstrate a cocktail effect, decreasing the overall side effects to the fetus, and with various therapeutics acting on different steps of inflammation.

Pentoxifylline (PTX) is a synthetic methylxanthine derivative that competitively inhibits phosphodiesterases with nonsteroidal immunomodulatory activities. Phosphodiesterase inhibition is believed to be associated with an anti-inflammatory effect, leading to decreased proinflammatory cytokine activity including tumor necrosis factor-alpha (TNF-α) and interferons (IFN)[8]. On this basis, PTX is presently under clinical investigation against neonatal sepsis, and a Cochrane meta-analysis has shown decreased sepsis-associated neonatal mortality with use of PTX as an adjunct to antibiotics[9]. A recent meta-analysis has also confirmed reduced mortality (relative risk, 0.50 and 95% confidence interval, 0.29 to 0.88) in sepsis following neonatal PTX treatment[10]. Therefore, maternal use of PTX for inflammation-driven pregnancy complications that might have effects on the fetus seems plausible, considering likely negligible toxicity to the preterm fetus.

In the sheep model, PTX was reported to decrease serum pro-inflammatory cytokines following experimentally induced endotoxemia[11]. Another experimental study revealed that PTX was partly effective against pre-eclamptic-like symptoms in ewes[12]. Considering these preliminary animal data, PTX can be considered to have fetal neuroprotective effects by mitigating IA inflammation. Moreover, IA injection of PTX can be hypothesized to deliver the drug in a target-specific manner to act on the fetal alveolar capillary bed and the gastrointestinal system by fetal swallowing.

The experimental ovine and caprine pregnancy models have been in use for translational research due to the physiological similarities with the human pregnancy, avoidance of multiple pregnancies with proper mating strategies, feasibility of IA administrations and sampling, and availability of adequate amount of fetal blood and tissues. Hence, preterm goat model may offer some advantages for experimental obstetric research.

The aim of the current study was to evaluate maternal oral and IA administration of PTX for the prevention of fetal inflammation and injury in a caprine model. We hypothesized that both oral and IA routes of PTX therapy were effective against inflammation-mediated fetal injury in the preterm period, particularly in the developing lung and brain tissues. To test this hypothesis, we used the small ruminant experimental model that utilized maternal granulocyte-colony stimulating factor (G-CSF) followed by high-dose IA endotoxin to aggravate intrauterine inflammation. We then evaluated various inflammatory, apoptotic, and injury parameters in the amniotic fluid, fetal blood plasma, placenta, fetal lungs, and fetal brain of the preterm goat fetuses.

Materials and Methods

Animal Material

Süleyman Demirel University Animal Experimentation Local Ethics Committee approved the study protocol (approval number: 15/03, date: 23.08.2011). Particular national regulations and principles of laboratory animal care were followed. A priori power analysis was conducted to test the difference between two independent group means for a 50%

decrease or increase in the mean, using a two-tailed test and an alpha of 0.05. Result showed that 4 animals in a group were required to achieve a power of 80%. Therefore, the experimental preterm goat model included 32 date-mated singleton pregnant hair goats (Capra hircus) with 8 groups (n=4 each) formed at day 100 of gestation (term pregnancy, approximately 150 days). The animals were reared on pasture and/or standard food, given water and mineral salts ad libitum, and were sheltered in a semi-open pen. All experiments were carried out at the Faculty of Veterinary Medicine, Mehmet Akif Ersoy University. Maternal age and prepregnancy body weight was 4-5 years and 40±5 kg, respectively.

Experimental Groups

The experiments were carried out in 2 phases. The first phase included validation of inflammation-mediated preterm fetal injury experimental model, including the vehicle control (group 1, n=4) and the positive control (group 2, n=4) groups. Details and results of these first 2 groups with proper validation of the novel modified experimental model initially defined by Watanabe et al.[13] have been described previously[14]. Recombinant G-CSF at a daily dose of 50 microg (dissolved in 2 mL normal saline) (Neupogen Roche, F. Hoffmann-La Roche Ltd, Basel, Switzerland) to induce low-grade maternal inflammation was injected into maternal jugular vein for 5 consecutive days (gestational days, 110-114) with controls receiving intrajugular normal saline. At gestational day 115, amniocentesis with a 20-gauge amniocentesis needle (EchoTip, Cook Medical, United States) was performed under ultrasound guidance (Echo Camera SSD-500, Aloka, Tokyo, Japan) with exclusion of inappropriate allantoic entry by the color and viscosity of the fluid[15]. Amniotic fluid, watery in consistency and pale amber in color was aspirated from the inner amniotic sac close to the fetus. Using the amniocentesis needle in the amniotic sac, high-dose (20 mg) IA endotoxin (lipopolysaccharides from Escherichia coli O55:B5, L 2880, Sigma-Aldrich, Missouri, USA) was used to induce IA and fetal inflammation in group 1, while the positive controls (group 2) received identical amount of IA normal saline through amniocentesis. Validation of the model with resultant necrotizing funisitis associated with abundant leukocyte infiltration leading to necrotic arc formation in the vascular wall of the umbilical vessel and secondary fetal brain injury was shown in all of the fetuses (n=4) in the experimental group (group 2, positive controls)[14]. Priming with maternal G-CSF followed by high-dose (20 mg) endotoxin closely mimics the FIRS, in which low-grade preclinical systemic maternal and IA inflammation is followed by a relatively abrupt insult that leads to fetal brain injury that is evident following endotoxin exposure[14].

The second phase of the experiments included treatment (PTX) groups to test the oral as well as IA efficacy and safety of antenatal PTX therapy. Group 3 and group 4 were designed to assess the prophylactic use of oral PTX at 2 different doses against subsequent inflammatory-driven fetal injury. Does in both of these groups (n=4 each) were started on oral PTX at day 100, and daily treatment continued for 15 days from gestation day 100 to 114 at a daily dose of 30 mg/kg maternal weight (low dose) and 60 mg/kg maternal weight (high dose) PTX, respectively. A mortar and pestle were used to grind commercially available tablets (Trental CR 600 mg film tablet, Sanofi Aventis, Istanbul, Turkey) into a uniform powder. After weighing on precision scales to calculate the dose, the PTX powder was dissolved in 10 mL of sterile water and administered to the does via an oral feeding catheter connected to a 10 mL-syringe. The does were observed for 10 minutes postadministration, and the procedure was repeated if the solution was not properly swallowed or spitted out by the animal. Animals in other treatment groups (groups 5-8) were given 10 mL of sterile water without PTX. We determined the daily low- and high-doses of oral PTX, depending on previous pharmacokinetic data from large animals[16,17]. Group 3 and 4 animals also received G-CSF (days 100-114) and IA endotoxin to induce fetal injury similar to positive controls.

Group 5 and group 6 aimed to examine the therapeutic effectiveness of single-use IA PTX at 2 different doses against concurrent inflammation-mediated fetal injury. Pregnant does in group 5 (n=4) and group 6 (n=4) received the 5-day G-CSF regimen and high-dose IA endotoxin as previously defined. Additionally at day 115, either single low-dose (400 mg/kg estimated fetal weight) or high-dose (800 mg/kg estimated fetal weight) PTX was injected into the amniotic fluid following endotoxin administration, using an amniocentesis needle under ultrasound guidance. Estimated fetal weight was typically considered as 1.5 kg, corresponding to 600 mg (30 mL) and 1.200 mg (60 mL) of IA single-dose administration of PTX (Trentilin ampoule 100 mg/5 mL, Santa Farma, Istanbul, Turkey). Dose calculation for IA administration depended on the assumption that around 2.5% of IA administered inert substances would be swallowed by the fetus within 1 hour[18], corresponding to 10 mg/kg fetal weight and 20 mg/kg fetal weight for the low- and high-dose regimens, respectively in line with the recommended oral pediatric doses for PTX[19].

The last 2 groups (group 7 and group 8) included use of IA PTX without intrauterine inflammation, i.e. non-administration of G-CSF and endotoxin. These groups assessed the safety and fetoplacental effects of IA administration of standalone PTX in the absence of intrauterine inflammation. Does in group 7 and group 8 received 2 mL of normal saline into the maternal jugular vein for 5 days followed by amniocentesis at gestational day 120 with PTX injections of 400 mg/kg estimated fetal weight (group 7) or 800 mg/kg estimated fetal weight (group 8) PTX.

Induction of Preterm Delivery

At gestational day 120 (0.80 gestation), preterm delivery was induced in all of the pregnant goats (n=32) by paralumbar cesarean section under epidural anesthesia with concurrent maternal sedation and incisional infiltration with local anesthetic[14,15]. Sedation with xylazine (0.25 mg/kg i.m.) and

sacrococcygeal epidural anesthesia into the sacrococcygeal space with a 6-cm, 20-gauge spinal needle using injection of 25 mg lidocaine hydrochloride and 0.016 mg epinephrine were performed. Then, the whole abdominal area was cleansed with 10% povidine iodine solution. A paralumbar skin incision of approximately 10 cm in length was used to reach the uterus, which was opened from its dorsal curvature with extension of the uterine incision using scissors as necessary. Before amniotomy, a 10 mL sterile syringe was used to aspirate the amniotic fluid for sampling. Then, the fetus and the placenta were delivered, and an intact placentome was dissected and sampled. The uterus was comprehensively lavaged with sterile saline solution to clean of all blood clots and membranes. Then, the uterus and the abdominal wall were closed with polyglactin 910 (Vicryl) interrupted sutures, and standard wound dressing was applied. Postpartum does were given systemic analgesic treatment with metamizole sodium and combined antibiotic treatment with 200 mg i.m. procaine penicillin and 250 mg i.m. dihydrostreptomycine sulfate.

The neonate kids were dried, weighed, and subjected to euthanasia with 50 mg/kg of intraperitoneal sodium thiopental (Pental Sodyum, IE Ulugay, Istanbul, Turkey) followed by transthoracic intracardiac blood sampling. Then, neonatal chest and skull were opened for en bloc dissection of the lung and brain. Parenchymal tissue from the lungs and white matter from the brains were sampled[14]. Tissue samples were fixed in 10% buffered formaldehyde and embedded into paraffin.

Evaluation of the Samples and Immunohistochemistry

Double-antibody sandwich enzyme-linked immunosorbent assay was used to evaluate interleukin-1 (IL-1), IL-4, IL-6, and TNF-α levels in the amniotic fluid and neonatal plasma samples, using commercial kits for goat serum (Eastbiopharm, Hangzhou, China). Results were evaluated at 450 nm, and optic density values were calculated and standardized accordingly.

Immunochemical staining on fetal lung, fetal brain and placental tissues were carried out using a routine streptavidine-biotin peroxidase technique[15]. After primary antibody incubation, streptoavidine peroxidase incubation of the slides for 20 minutes was carried out followed by washing with phosphate-buffered saline (PBS) biotinilated Abs for 30 min. Then, PBS was used to rinse the slides, and a peroxidase substrate solution containing 3,3′-Diaminobenzidine (DAB Substrate Kit, ab94665, Abcam, UK) was used for 5 minutes of incubation. Then, rinsing with distilled water, counterstaining with hematoxylin, dehydration, and mounting of the slides were performed.

All tissues were immunostained for IL-1, IL-4, IL-6, TNF-α, caspase 3, caspase 5, caspase 7, cyclo-oxygenase-1 (COX-1), COX-2, IFN-alpha (IFN-α), and IFN-beta (IFN-β) (Abcam, UK). Additional immunostaining included surfactant proteins (SP) A, B, C, and D (Santa Cruz Biotechnology Inc., USA), and prosurfactant protein B (pro-SP-B, Abcam, UK) for the lung tissues and neuron specific enolase (NSE, Abcam, UK), glial fibrillary acidic protein (GFAP, Abcam, UK), vimentin (Abcam, UK), anti-neurofilament protein (NFP, Abcam, UK), and anti-myelin basic protein (MBP, Abcam, UK) for the brain tissues.

Degree of immunostaining was assessed by the pathologists blindly, concerning experimental groups with an arbitrary visual scale that graded the immunoreaction as 0, no staining; 1, weak staining; 2, moderate staining; and 3, diffuse staining. For semiquantitative evaluations, Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) fitted with a light microscope (Olympus CX41) was utilized.

Statistical Analysis

Data were expressed as mean and standard deviations. Kruskal-Wallis H test and Mann-Whitney U test were used for multiple and binary comparisons, respectively. Statistical significance was set at $p<0.05$. Kruskal-Wallis H test p-values were shown in the tables, whereas Mann-Whitney U test results for between-group comparisons were given in the text in more detail.

Results

The mean neonatal weight was 1.453±260 g, and was similar across the groups (p=0.62). Similarly, the fetal gender was distributed equally across the groups (p=0.09). Comparisons of group 1 (vehicle control) and group 2 (fetal injury model by maternal G-CSF and high-dose IA endotoxin) have been specified in detail in our previous publication[14] with validation of the current model of intrauterine inflammation. Briefly, maternal G-CSF and IA endotoxin led to increased IL-1, TNF-α, IFN-α, and IFN-β, COX-1, COX-2, caspase 3, 5, and 7, and reflex increase in IL-4 and IL-6 in all of the evaluated tissues along with decreased pulmonary SP-A, SP-B, SP-C, SP-D, pro-SP-B, and decreased brain vimentin, neuron specific enolase (NSE), neurofilament protein (NFP), GFAP, and myelin basic protein (MBP) expressions.

Amniotic Fluid Inflammatory Markers

Data on amniotic fluid sampled at preterm cesarean delivery are given in Table 1 and summarized in Figure 1. Both low-and high-dose oral PTX regimens significantly lowered IA IL and TNF-α levels compared to the model group (p=0.03 for both comparisons), However, reflex increase in immunomodulatory interleukins was more efficiently suppressed with high-dose oral PTX (group 4) compared to the low-dose (group 3), considering IL-4 and IL-6 expressions (p=0.04 for all comparisons). High-dose oral administration was able to partially reverse IA inflammation with comparable IL-1 (p=0.15) and IL-6 (p=0.07) values across group 1 (vehicle controls) and group 4.

Both low-and high-dose IA PTX were also effective in reducing IA inflammation with decreased IA ILs and TNF-α compared to group 2 (p=0.03 for all comparisons except p=0.07 for low-dose IA PTX). The anti-inflammatory effect seemed similar between two IA doses ($p>0.05$ for all comparisons across group 5 and group 6). Despite this, IA PTX administrations were not able to completely reverse the IA inflammation, since comparisons of

Table 1. Comparisons of amniotic fluid and neonatal plasma inflammatory markers between experimental groups (n=4 in each group) in preterm caprine pregnancies

Inflammatory marker	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	p
Amniotic fluid									
Interleukin-1 (pg/mL)	95.0±8.2	217.5±27.3	145.0±41.6	107.7±8.1	166.0±50.9	120.0±12.3	101.0±7.2	94.0±13.9	0.017
Interleukin-4 (pg/mL)	110.7±33.2	505.5±70.2	380.0±21.3	299.0±3.5	410.0±47.5	395.0±43.9	110.0±19.4	175.0±35.1	0.006
Interleukin-6 (pg/mL)	63.7±15.0	196.2±37.7	109.0±10.4	86.3±8.1	133.0±4.0	113.0±16.1	109.0±11.9	92.0±10.1	0.008
TNF-alpha (pg/mL)	112.7±5.4	299.7±5.2	150.0±21.5	133.3±11.5	195.0±12.2	190.0±13.4	110.0±69.1	109.0±40.6	0.009
Neonatal plasma									
Interleukin-1 (pg/mL)	70.0±20.3	142.2±20.0	115.0±7.0	93.3±11.5	132.0±15.6	100.0±6.2	80.1±9.1	75.0±8.2	0.008
Interleukin-4 (pg/mL)	87.5±10.4	485.2±489	325.0±27.0	259.3±28.9	388.0±31.2	369.0±40.0	159.0±27.4	149.0±46.5	0.006
Interleukin-6 (pg/mL)	44.7±3.0	156.0±30.1	68.0±4.6	59.0±3.5	112.0±14.0	105.0±4.6	109.0±9.4	75.0±17.7	0.007
TNF-alpha (pg/mL)	79.1±14.2	160.5±22.1	120.0±24.0	110.3±8.1	159.0±47.8	129.0±20.9	90.0±23.5	85.0±5.3	0.012

Group 1: Negative controls, Group 2: Positive controls with inflammation-mediated fetal injury, Group 3: Low-dose oral pentoxifylline + fetal injury, Group 4: High-dose oral pentoxifylline + fetal injury; Group 5: Low-dose intra-amniotic pentoxifylline + fetal injury, Group 6: High-dose intra-amniotic pentoxifylline + fetal injury, Group 7: Low-dose intra-amniotic pentoxifylline without fetal injury, Group 8: High-dose intra-amniotic pentoxifylline without fetal injury. Data are expressed as mean ± standard deviation. TNF: Tumor necrosis factor

all parameters between IA PTX treatment groups and vehicle controls showed statistically significant differences (p=0.03 for all). Standalone IA PTX treatment (group 7 and group 8) was generally associated with similar IA inflammatory marker values (p>0.05) except increased IL-6 (p=0.02) with low-dose PTX (group 7) compared to group 1 controls.

The current data underpinned the anti-inflammatory effects of both prophylactic oral and therapeutic IA PTX, although high-dose oral protocol showed a more robust activity to alleviate endotoxin-induced IA inflammation (Figure 1).

Neonatal Plasma Inflammatory Markers

Comparisons of the studied inflammatory parameters in the neonatal blood plasma obtained following preterm delivery are summarized in Table 1 and Figure 1. Low-dose oral PTX was associated with decreased IL-1, -4, and -6 levels in the neonatal plasma (p=0.03 for all), although TNF-α was unchanged (p=0.07), when compared with vehicle controls (Table 1). High-dose oral PTX seemed more effective in reducing circulating inflammatory markers, with all of the studied ILs and TNF-α significantly less than that of group 2 (p=0.03 for all comparisons). However, only IL-1 measurements after high-dose oral PTX were similar (p=0.07) to normal controls, indicating a partial reversal of inflammation following the high-dose oral regimen.

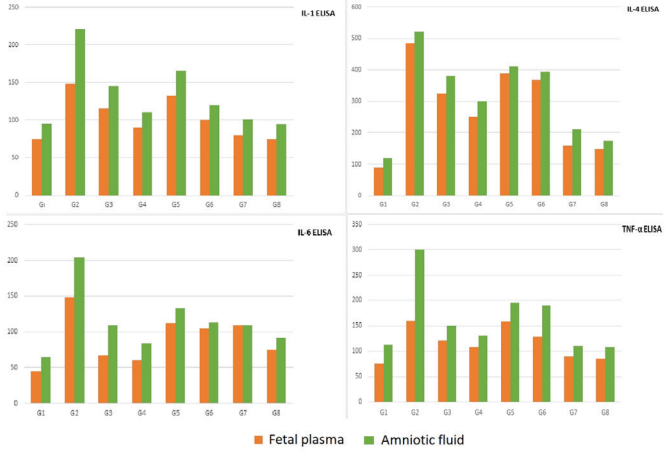

Figure 1. Fetal blood plasma and amniotic fluid interleukin-1, interleukin-4, interleukin-6, and tumor necrosis factor-alpha measurements obtained at preterm delivery in experimental study groups

For IA treatment with PTX, high-dose (group 6) decreased all of the studied neonatal plasma ILs (p=0.03 for all comparisons), but not TNF-α (p=0.07) compared to endotoxin controls (group 2). However, only mean IL-1 level was similar (p=0.07) to that of group 1 controls, indicating a partial reversal of inflammation with the high-dose administration. Low-dose IA PTX (group 5) was less effective with only IL-4 levels significantly decreased (p=0.04) in the neonatal plasma compared to endotoxin controls. Despite these positive effects, standalone IA PTX was associated with increased IL-6 plasma measurements following both low-dose and high-dose IA PTX (p=0.02 and p=0.03, respectively) compared to vehicle controls.

As a result, both high-dose oral and high-dose IA PTX effectively reduced the studied inflammatory markers in the neonatal plasma, but did not completely reverse the fetal inflammation back to control levels. A possible adverse effect of IA PTX may be stimulation of an immune reaction through IL-6 in the fetus (Figure 1).

Placental Immunohistochemical Findings

Table 2 summarizes comparisons of placental immunostaining intensities between the study groups. Group 2 (model) was associated with profound placental inflammation and apoptosis, as previously reported[14]. Low-dose oral PTX (group

Table 2. Comparisons of placental immunostaining intensities between experimental groups (n=4 in each group) of preterm caprine pregnancies

Marker	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	p
Interleukin-1	0.25±0.5	3.0±0	1.3±0.6	0.3±0.6	1.0±0.0	1.3±1.5	1.0±0.8	1.3±1.1	0.012
Interleukin-4	0.5±0.6	2.75±0.5	1.0±1.0	0.7±0.6	1.3±1.1	1.7±1.5	1.2±0.9	1.3±1.1	0.035
Interleukin-6	0.25±0.5	2.75±0.5	1.0±1.0	0.3±0.6	0.7±1.1	1.3±1.5	1.5±1.0	1.0±1.0	0.026
Interferon-alpha	0.25±0.5	2.75±0.5	1.3±1.5	0.7±0.6	1.0±0.0	1.0±1.7	1.0±0.5	1.0±1.0	0.044
Interferon-beta	0.25±0.5	2.75±0.5	0.7±1.1	0.7±0.6	2.3±0.6	1.0±1.7	1.2±0.5	0.3±0.6	0.032
Tumor necrosis factor-alpha	0.25±0.5	2.5±1.0	1.0±1.7	0.3±0.6	1.3±1.5	1.7±1.5	1.0±0.8	1.0±1.0	0.081
Cyclooxygenase-1	0.75±0.5	2.5±0.6	1.3±1.1	0.7±1.1	2.0±1.7	1.0±1.0	1.0±0.8	1.7±0.6	0.071
Cyclooxygenase-2	0.0±0.0	2.75±0.5	0.7±1.1	1.0±0.0	0.7±1.1	1.7±0.6	1.0±1.1	0.7±1.1	0.014
Caspase 3	0.25±0.5	3.0±0.0	1.0±1.0	0.3±0.6	1.3±1.5	1.3±1.5	1.0±0.0	1.3±0.6	0.021
Caspase 5	0.25±0.5	2.5±0.6	0.7±1.1	0.3±0.6	0.7±1.1	1.7±1.6	1.0±1.1	1.0±1.0	0.036
Caspase 7	0.25±0.5	2.5±0.6	1.7±0.6	1.0±0.0	1.3±0.6	1.0±1.0	1.5±0.6	1.3±0.6	0.012

Group 1: Negative controls, Group 2: Positive controls with inflammation-mediated fetal injury, Group 3: Low-dose oral pentoxifylline + fetal injury, Group 4: High-dose oral pentoxifylline + fetal injury, Group 5: Low-dose intra-amniotic pentoxifylline + fetal injury, Group 6: High-dose intra-amniotic pentoxifylline + fetal injury, Group 7: Low-dose intra-amniotic pentoxifylline without fetal injury, Group 8: High-dose intra-amniotic pentoxifylline without fetal injury. Data are expressed as mean ± standard deviation. Staining intensity was graded as 0, negative; 1, weak staining; 2, moderate staining; and 3, diffuse staining

3) partially reversed the findings, as shown by decreased IL-1 (p=0.02), IL-6 (p=0.04), caspase 3 (p=0.02), and COX2 (p=0.04) expressions compared to endotoxin exposed controls (group 2). High-dose oral PTX (group 4) alleviated placental inflammation and apoptosis to a great extent with return to baseline expressions (p>0.05) except COX2 (p=0.01 for group 1 and group 4 comparison).

Although low-dose IA PTX (group 5) led to decreased expressions of some inflammatory parameters in the placenta (IL-1, IL-6, IFN-α, and COX2 with p=0.01, p=0.04, p=0.04, and p=0.04, respectively) compared to positive controls, high-dose IA PTX (group 6) seemed ineffective (p>0.05 for all comparisons between group 2 and group 6). Standalone low- and high-dose IA PTX was associated with increased caspase 3 (p=0.04 and p=0.05, respectively) and caspase 7 (p=0.03 and p=0.05, respectively) expressions in the placenta, indicating a possible apoptotic process in the placenta following PTX injection into the amniotic fluid (Figure 2).

These results revealed that prophylactic oral PTX prevented placental inflammation and apoptosis, and high-dose oral regimen was probably more effective with return to baseline levels except COX2. On the other hand, IA PTX was less effective and may be associated with placental apoptosis.

Fetal Brain Immunohistochemical Findings

Table 3 summarizes the immunostaining intensities of the fetal brain white matter. As previously shown[14], inflammatory and apoptotic markers were increased (p<0.05), leading to fetal brain injury apparent with decreased NSE, NFP, GFP, and MBP staining in endotoxin-exposed positive controls (group 2). Low-dose oral PTX (group 3) was associated with decreased IL-1 (p=0.02), IFN-β (p=0.03), caspase 3 (p=0.03), COX1 (p=0.02),

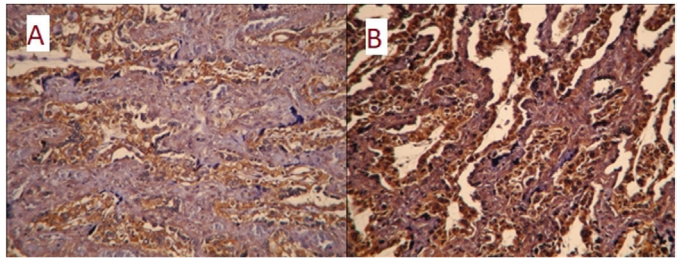

Figure 2. Placental caspase-7 immunoreaction in (A) group 6 and (B) group 7 that received intra-amniotic pentoxifylline with or without intra-amniotic endotoxin, respectively. Administration of pentoxifylline directly into the amniotic fluid could trigger apoptosis in the placenta

and increased NSE (p=0.03) staining compared to positive controls (group 2). On the other hand, high-dose oral PTX (group 4) had a more profound effect, leading to significantly improved expressions (p<0.05) of all of the parameters except COX-2 (p=0.06), GFAP (p=0.06), and MBP (p=0.06). There were no significant differences (p>0.05 for all comparisons) between group 4 and vehicle controls (group 1), indicating reversal of the brain injury with high-dose oral PTX.

Low-dose IA PTX (group 5) led to decreased IL-1 (p=0.018), IFN-α (p=0.04), IFN-β (p=0.04), and TNF-α (p=0.02) immunostaining in the fetal brain compared to endotoxin controls (group 2). Following high-dose IA PTX (group 6), only IL-1 (p=0.02), and IFN-β (p=0.04) staining were reduced. Low- and high-dose IA PTX (group 5 and group 6) were similar (p>0.05 for all comparisons) considering all parameters. Low-dose PTX administration into the amniotic fluid without fetal injury (group 7) was associated with increased caspase 3 (p=0.02) and caspase 5 (p=0.04), and decreased NSE (p=0.02),

Table 3. Comparisons of fetal brain white matter immunostaining intensities between experimental groups (n=4 in each group) of preterm kids

Marker	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	p
Interleukin-1	0.25±0.5	3.0±0	1.7±0.6	0.3±0.6	1.3±0.6	1.3±0.6	1.2±0.9	1.0±1.0	0.010
Interleukin-4	0.25±0.5	2.25±0.5	0.7±0.6	0.7±0.6	1.0±1.0	1.0±1.7	1.2±0.9	1.0±1.0	0.023
Interleukin-6	0.25±0.5	2.5±1.0	0.7±1.1	0.3±0.6	0.7±1.1	1.0±1.0	1.0±0.8	0.7±1.1	0.053
Interferon-alpha	0.25±0.5	2.75±0.5	1.3±1.5	0.3±0.6	1.0±1.0	1.0±1.7	1.0±0.8	0.7±0.6	0.041
Interferon-beta	0.25±0.5	3.0±0	2.0±1.0	0.3±0.6	1.0±0.0	1.0±1.7	1.2±0.5	0.3±0.6	0.013
Tumor necrosis factor-alpha	0.25±0.5	2.75±0.5	0.7±0.6	0.3±0.6	0.7±1.1	1.0±1.0	1.5±1.3	1.0±1.0	0.024
Cyclooxygenase-1	0.5±0.6	2.5±0.6	1.0±0.0	0.3±0.6	1.3±1.1	1.3±1.1	1.0±0.8	1.7±0.6	0.018
Cyclooxygenase-2	0.25±0.5	2.25±0.9	1.3±1.5	0.7±0.6	1.3±1.5	1.3±1.5	0.5±0.6	1.0±1.0	0.088
Caspase 3	0.25±0.5	2.75±0.5	0.7±0.6	0.7±0.6	1.7±0.6	2.4±1.1	1.7±0.5	1.0±1.0	0.023
Caspase 5	0.25±0.5	2.25±0.5	1.3±0.6	0.7±0.6	1.0±1.0	1.7±0.6	1.2±0.5	1.0±1.0	0.018
Caspase 7	0.25±0.5	2.25±0.5	1.3±1.1	1.0±0.0	1.0±1.0	1.0±1.7	0.7±0.9	1.0±1.0	0.032
Vimentin	3.0±0	1.5±0.6	2.0±1.0	3.0±0	2.0±1.7	2.3±1.1	2.0±1.1	1.7±0.6	0.021
Neuron specific enolase	3.0±0	0.5±0.6	2.3±0.6	3.0±0	0.7±1.1	0.7±1.1	1.2±0.9	1.3±0.6	0.009
Neurofilament protein	3.0±0	1.0±0.8	2.0±1.0	3.0±0	2.0±1.0	1.0±0.0	1.7±1.5	2.3±0.6	0.017
Glial fibrillary acidic protein	3.0±0	1.5±1.0	2.7±0.6	3.0±0	1.0±1.0	2.0±1.0	2.2±0.9	2.3±0.6	0.062
Myelin basic protein	3.0±0	1.5±1.0	2.7±0.6	3.0±0	2.0±1.0	2.0±1.0	2.2±0.9	2.7±0.6	0.062

Group 1: Negative controls, Group 2: Positive controls with inflammation-mediated fetal injury, Group 3: Low-dose oral pentoxifylline + fetal injury, Group 4: High-dose oral pentoxifylline + fetal injury, Group 5: Low-dose intra-amniotic pentoxifylline + fetal injury, Group 6: High-dose intra-amniotic pentoxifylline + fetal injury, Group 7: Low-dose intra-amniotic pentoxifylline without fetal injury, Group 8: High-dose intra-amniotic pentoxifylline without fetal injury. Data are expressed as mean ± standard deviation. Staining intensity was graded as 0, negative; 1, weak staining; 2, moderate staining; and 3, diffuse staining

whereas high-dose (group 8) led to decreased vimentin and NSE (p=0.02 for both) compared to vehicle controls (group 1). Therefore, high-dose oral PTX seemed more effective than low-dose oral PTX against fetal brain injury. The results also indicated some anti-inflammatory effect of IA PTX on the preterm fetal brain, although PTX might lead to increased white matter apoptosis and neuronal injury, when injected into the amniotic fluid.

Fetal Pulmonary Immunohistochemical Findings

Table 4 shows the immunostaining intensities of the fetal pulmonary parenchyma. As expected, inflammatory and apoptotic parameters were increased, whereas surfactants were decreased in the model group (group 2). Low-dose oral PTX (group 3) alleviated IL-1 (p=0.02), IL-4 (p=0.04), IFN-α (p=0.02), IFN-β (p=0.03), caspase 3 (p=0.03), caspase 5 (p=0.03), and COX1 (p=0.02) expressions compared to positive controls (group 2). High-dose oral PTX (group 4) was more effective, as shown by amelioration of all lung parameters to baseline (group 1 control) levels (p>0.05 for all comparisons) and significantly decreased inflammation and apoptosis (p<0.05 for all) except COX2 staining (p=0.07) with increased surfactant protein levels (p=0.02 for all).

IA PTX was generally associated with improved fetal pulmonary inflammation; however, lacked any positive effects on apoptosis and surfactant synthesis. Both low- and high-dose IA PTX (group 5 and group 6) decreased pulmonary IL-1 (p=0.02 for both doses), reflex increase in IL-4 (p=0.02 for both doses), and IFN-α staining (p=0.01 and p=0.02, respectively) compared to endotoxin-exposed controls (group 2). High-dose was also associated with decreased pulmonary COX1 expressions (p=0.02). No significant differences were present for other parameters, when group 5 or 6 were compared with group 2. Standalone (i.e. without endotoxin) IA PTX at low (group 7) or high (group 8) doses did not generally alter immunostaining of the studied parameters (p>0.05 for all comparisons with group 1 controls), demonstrating that PTX did not have any unwanted effects on fetal lungs after IA administration.

Overall, high-dose oral PTX was the most effective treatment modality against inflammation-driven preterm fetal pulmonary injury with return to baseline expressions except COX2.

Discussion

In the current study, we tested the therapeutic efficacy of oral and IA PTX administrations against inflammation-mediated placental and fetal injury in the preterm goat model. Oral PTX was given prophylactically (i.e. before IA endotoxin injection), whereas IA PTX was administered concomitantly with endotoxin. Two different doses labeled as low- and high-dose treatments were evaluated for both the oral and IA ways of administration. Overall data indicate high-dose oral (60 mg/kg maternal weight daily) use of PTX for 15 days prior to IA

Table 4. Comparisons of fetal pulmonary parenchymal immunostaining intensities between experimental groups (n=4 in each group) of preterm kids

Marker	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	p value
Interleukin-1	0.25±0.5	3.0±0	0.7±0.6	0.7±0.6	1.3±0.6	0.7±1.1	0.7±0.5	0.7±1.1	0.022
Interleukin-4	0.25±0.5	2.75±0.5	0.7±1.1	0.3±0.6	1.0±1.0	1.0±1.0	1.5±1.0	1.3±1.1	0.030
Interleukin-6	0.5±0.6	2.5±0.6	1.0±1.0	0.7±0.6	0.7±0.6	0.7±1.1	1.0±1.1	0.3±0.6	0.040
Interferon-alpha	0.25±0.5	3.0±0	1.3±0.6	0.7±0.6	1.0±0.0	1.3±1.1	1.7±1.3	0.7±0.6	0.013
Interferon-beta	0.25±0.5	2.75±0.5	2.0±1.0	0.3±0.6	2.0±1.0	2.0±1.7	1.0±0.8	0.3±0.6	0.017
Tumor necrosis factor-alpha	0.25±0.5	2.75±0.5	0.7±0.6	0.7±0.6	1.3±0.6	2.0±1.0	1.5±1.3	2.0±0.0	0.023
Cyclooxygenase-1	0.25±0.5	2.75±0.5	1.0±0.0	0.3±0.6	2.0±1.0	1.0±0.0	1.2±1.2	0.7±0.6	0.014
Cyclooxygenase-2	0.75±0.5	2.5±1.0	0.7±1.1	1.0±0.0	1.3±1.5	0.7±1.1	1.2±1.5	1.0±1.0	0.095
Caspase 3	0.25±0.5	2.5±0.6	0.7±0.6	0.3±0.6	1.0±1.0	1.7±1.5	0.5±0.6	1.0±1.0	0.024
Caspase 5	0.25±0.5	2.25±0.5	0.3±0.6	0.7±0.6	1.0±1.0	1.3±1.1	0.7±0.9	1.0±1.0	0.024
Caspase 7	0.25±0.5	2.5±1.0	1.0±0.0	0.3±0.6	1.3±0.6	2.0±0.0	1.2±0.9	1.7±0.6	0.040
Surfactant protein A	2.5±0.6	0.25±0.5	1.3±0.6	2.3±0.6	1.7±1.1	0.7±0.6	1.2±1.2	2.3±0.6	0.016
Surfactant protein B	2.5±0.6	0.5±0.6	1.0±1.0	2.7±0.6	0.7±1.1	1.0±1.7	1.5±1.3	2.0±1.0	0.023
Surfactant protein C	2.75±0.5	0.25±0.5	1.0±1.0	3.0±0	0.3±0.6	0.7±1.1	1.2±1.2	2.3±0.6	0.012
Surfactant protein D	3.0±0	0.75±0.9	1.0±1.7	3.0±0	1.3±1.1	1.0±1.0	2.0±1.4	2.3±1.2	0.026
Pro-surfactant protein B	3.0±0	0.5±0.6	1.0±1.0	3.0±0	1.0±1.7	0.7±0.6	2.0±0.8	2.7±0.6	0.009

Group 1: Negative controls, Group 2: Positive controls with inflammation-mediated fetal injury, Group 3: Low-dose oral pentoxifylline + fetal injury, Group 4: High-dose oral pentoxifylline + fetal injury, Group 5: Low-dose intra-amniotic pentoxifylline + fetal injury, Group 6: High-dose intra-amniotic pentoxifylline + fetal injury, Group 7: Low-dose intra-amniotic pentoxifylline without fetal injury, Group 8: High-dose intra-amniotic pentoxifylline without fetal injury. Data are expressed as mean ± standard deviation. Staining intensity was graded as 0, negative; 1, weak staining; 2, moderate staining; and 3, diffuse staining

endotoxin insult as the most effective protective therapeutic option against inflammation-driven fetal injury. Although PTX when given into the amniotic fluid had some potential to decrease the IA inflammation, some adverse effects such as increased apoptosis in the placenta and fetal brain secondary to stimulation of an immune or toxic reaction is a concern that may restrict use of IA PTX for preterm fetal neuroprotection.

There are some potential explanations why oral PTX was more effective in our design. Since the oral protocol was prophylactic in nature with repeated daily doses, the neuroprotective effects of PTX might have become more apparent before the inflammatory insult. This may imply that PTX is generally active as a preventive therapy, when given relatively early in the inflammation-driven preterm birth model with limited action following delayed administration. Repeated doses of PTX instead of a single-dose may be required for relevant action, as well. However, maternal and placental pharmacokinetics of PTX may also play a role. Oral PTX administered maternally is metabolized into at least 7 nonconjugated metabolites by the liver, and the main active metabolite called lisofylline has been shown to have anti-inflammatory and antifibrotic activity[20,21]. Moreover, the anti-inflammatory actions of both PTX and lisofylline were attributed to 8-oxo derivatives rather than the parent forms[22]. Since administration of PTX directly into the amniotic fluid will not enable hepatic metabolization of PTX into active forms, IA drug delivery may have inadequate anti-inflammatory and fetal neuroprotective actions. Furthermore, it can be speculated that the maternal liver and possibly the placenta "detoxify" the parent compound into more active and less fetotoxic compounds. Evidence in favor of such an effect can be adverse fetal reactions we encountered in our design, including increased apoptosis in fetal brain following IA PTX administrations in pregnancies without intrauterine inflammation. In summary, oral PTX administered to risk-groups for preterm delivery should be the preferred way of administration for fetal neuroprotection.

Experimental data on use of PTX for inflammation-induced adverse effects in the placenta are scarce. In a tissue culture model that used second trimester human placentas treated with endotoxin and PTX, placental expression and production of inflammatory markers such as IL-1, TNF-α, and IFN were shown to decrease with administration of PTX[23]. These *ex vivo* results are in agreement with *in vivo* data from our experiments, affirming the anti-inflammatory actions of PTX on the placenta. Some previous animal studies have also evaluated the placental and fetal effects of maternal PTX in the endotoxin-induced intrauterine inflammation models. PTX was reported to mitigate endotoxin-induced up-regulation of placental heme oxygenase-1 in pregnant mice[24]. PTX was also shown to decrease embryo resorption, fetal mortality, and fetal

growth restriction following lipopolysaccharide injections to pregnant mice[25,26]. However, these positive effects were only partially replicated in a rabbit model. In pregnant rabbits given intrauterine endotoxin, animals that received PTX 20 mg/kg/day in 3 divided doses had similar preterm delivery rates, despite prolonged time until fetal death compared to controls[27]. On the other hand, in experimentally induced equine placentitis using intracervical inoculation of ***Streptococcus equi***, 17 mg/kg daily maternal dose of PTX given orally from the onset of clinical signs to delivery was associated with improved viability of foals and negative fetal bacterial cultures[28]. Depending on these results in association with our current data, oral maternal PTX treatment given for a longer period, particularly before placental and fetal injury commences seems more effective to alleviate subsequent intrauterine inflammation.

In the English literature, we were able to identify only one experimental study that specifically evaluated the fetal neuroprotective effects of antenatally administered PTX. This study by Dilek et al.[29] used intraperitoneal endotoxin to induce fetal injury in pregnant rats, and 3 doses (60 mg/kg) of maternally injected PTX before term delivery was associated with decreased apoptosis and MBP immunostaining in periventricular white matter of the pups. The results imply that PTX is a potential neuroprotective agent against fetal term brain injury[29]. Our data from a phylogenetically diverse animal model in the early preterm period with numerous inflammatory and neuronal injury markers elaborate these previous findings.

In summary, previous experimental data show that prophylactic maternal PTX treatment may decrease placental and fetal brain injury following an intrauterine inflammatory insult, but probably do not prevent preterm delivery. Although we did not specifically address the timing of preterm delivery in our experiments, our results generally elaborate these findings and support use of maternal PTX as a fetal neuroprotective agent, particularly for pregnancies at risk for preterm delivery. Moreover, we showed the efficacy of oral daily doses at 60 mg/kg, which is clinically more feasible than parenteral injections.

We could identify only one clinical study on the use of antenatal PTX in human pregnancies. The randomized prospective trial by Lauterbach et al.[30] included 96 women between 23-34 weeks of gestation with imminent preterm delivery, randomized to 800 mg/day PTX (n=43) and controls (n=53) for 3 weeks or until delivery, with all the women receiving standard beta-mimetic tocolysis, corticosteroids for fetal lung maturation, and magnesium sulfate for neuroprotection. The cerebroplacental ratio at week 3 of treatment was found to be significantly higher in the treatment arm. Moreover, composite neonatal outcome (intraventricular bleeding, periventricular leukomalacia, and neonatal mortality) was lower in the PTX arm, although time to delivery was similar. Our results are principally in line with the findings from this pilot clinical study[30]. We did not specifically evaluate fetal Doppler studies or neonatal mortality. However, our results similarly imply that PTX given for at least 2 weeks has fetal neuroprotective effects. Lauterbach et al.[30] administered half of the daily 800 mg dose as an intravenous infusion, and the rest 400 mg as oral tablet. Considering the dose scheme in our study that corresponds to higher oral doses than that of Lauterbach et al.[30], the necessity and feasibility of additional daily intravenous administrations are questionable. Therefore, future clinical studies evaluating fetal neuroprotective effects of prophylactic antenatal PTX therapy can focus on relatively low (800-1600 mg) daily oral doses of PTX without any parenteral administrations, which necessities admission to the hospital.

Study Limitations

There were certain limitations of the current experimental study. Although various tissue parameters for inflammation, apoptosis, and tissue injury were studied by immunohistochemistry, additional methods such as genetic expressions and Western blot were not evaluated. Since there is positive experimental and clinical evidence in favor of safety of oral PTX use during the third-trimester of pregnancy, our design did not include standalone oral PTX experimental groups. There was also no oral placebo group. However, addition of more experimental groups into the present design would be unethical. C-reactive protein and white blood cell count and differential in fetal blood samples were not measured, as interleukins and TNF-α were considered more specific for fetal inflammatory response. The current design was also unable to reveal data on temporal changes of the studied parameters. In our validated model, preterm birth was induced 5 days after IA endotoxin, a time period supposed to be most suitable for evaluation of fetal inflammatory response. However, we do not have data on other time points, including postpartum alterations, since newborn kids were euthanized after delivery to illustrate specifically ***in utero*** tissue responses. The present experimental model cannot also distinguish whether the experimental IA inflammation created in the intervention groups in the study essentially caused spontaneous preterm birth, since preterm delivery was induced iatrogenically to retrieve fetal and placental samples. Finally, CP is multifactorial condition that can occur during the intrapartum period, during, or after delivery. The current data do not explicitly reveal information on preterm birth related to CP and/or related complications, and are restricted to experimental fetal injury in the preterm period with lack of analysis of mid- and long-term results of CP.

Conclusion

Maternal PTX given orally before inflammation-mediated preterm delivery in a prophylactic fashion alleviates fetal inflammatory response and may exhibit fetal neuroprotective effects in the caprine model. Considering the favorable fetal safety profile of PTX during the third trimester of pregnancy, clinical studies evaluating its antenatal use in imminent preterm delivery against subsequent development of possible brain injury are warranted.

Ethics

Ethics Committee Approval: The study protocol was subject to animal ethics committee approval by Süleyman Demirel University Animal Experimentation Local Ethics Committee (approval date and no, 23.08.2011/03).

Informed Consent: Experimental study.

Authorship Contributions

Concept: M.S., A.K., Ö.Ö., M.H., D.K., A.A., O.Ö. Design: M.S., A.K., D.K. Data Collection or Processing: M.S., A.K., Ö.Ö., M.H., D.K., A.A., O.Ö. Analysis or Interpretation: M.S., Ö.Ö, M.H, O.Ö. Literature Search: M.S., A.F, O.Ö., Writing: M.S., Ö.Ö.

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The relationship between isolated pes equinovarus and aneuploidies and perinatal outcomes: Results of a tertiary center

İzole pes ekinovarus ile anöploidiler arasında ilişki ve perinatal sonuçlar: Tersiyer tek merkez sonuçları

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Abstract

Objective: Congenital pes equinovarus (PEV) is the most common congenital deformity of the foot, characterized by plantar flexion with a frequency of 0.2-0.3%. It can be diagnosed from the 12th week of pregnancy. Non-isolated cases tend to be syndromic and complex. We aimed to evaluate the results of perinatally diagnosed isolated PEV.

Materials and Methods: This was a retrospective cohort study conducted between March 2015-March 2020. Women who presented for fetal anomaly screening or were referred due to any suspected fetal anomaly were subjected to detailed fetal anomaly scans and checked for the presence of PEV. Karyotype analysis was discussed for patients with PEV. Pregnancy termination was recommended for those with chromosomal/life-threatening anomalies. The diagnosis was confirmed by postnatal examination/autopsy. Postnatal diagnosis was accepted as false-positive in those with no PEV.

Results: One-hundred thirty-eight patients were found to have PEV, 41 (29.7%) of which were isolated. In the isolated group, the false-positive rate in the first trimester was significantly higher compared with the second trimester, 50%/15.3%, respectively (p<0.05). Chromosomal anomalies were detected in 2 (4.8%) patients in the isolated group. Termination was performed to 1 (2.4%) patients due to trisomy 21. In the non-isolated group, chromosomal anomalies were detected in 13 (13.4%) patients, and termination was recommended. Termination was also recommended to 18 (18.5%) patients due to anomalies incompatible with life. In the postnatal evaluation, the surgical treatment rate in the isolated/non-isolated groups was 6%/39.7% (p<0.05).

Conclusion: When PEV is diagnosed, detailed fetal anomaly screening must be performed, patients should be informed about the chromosomal anomaly risk. High false-positive rates in the first trimester should be kept in mind for diagnosis. Karyotype analysis should be recommended also to isolated cases. It should be remembered that some neuromuscular/skeletal system anomalies may occur for the first time in the postnatal period in isolated cases.

Keywords: Clubfoot, Down syndrome, diagnostic imaging, karyotyping

Öz

Amaç: Konjenital pes ekinovarus (PEV), ayağın en sık görülen konjenital deformitesidir, aşırı plantar fleksiyon ile karakterizedir ve 1000 canlı doğumda 2-3 sıklıkla görülür. Gebeliğin 12. haftasından itibaren teşhis etmek mümkündür. İzole olmayan olgular sendromik ve daha karmaşık olma eğilimindedir. Çalışmamızda, perinatal dönemde teşhis edilen izole PEV olgularının sonuçlarını değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Çalışmamız Mart 2015-Mart 2020 tarihleri arasında yürütülen retrospektif kohort çalışmasıdır. Rutin fetal anomali taraması için başvuran veya herhangi şüpheli fetal anomali nedeniyle sevk edilen kadınlar, ayrıntılı bir fetal anomali taramasına tabi tutuldu, PEV varlığı ve eşlik eden anomaliler açısından kontrol edildi. PEV saptanan hastalarla karyotip analizi tartışıldı. Kromozom anomalisi veya hayatı tehdit eden anomalisi olanlara gebelik terminasyonu önerildi. Tanı postnatal muayene veya otopsi ile doğrulandı. Postnatal PEV saptanmayanlarda tanı yanlış pozitif olarak kabul edildi.

Bulgular: Yüz otuz sekiz hastada PEV saptandı. Kırk biri (%29,7) izole idi. İzole grup içinde, ilk trimesterde yanlış pozitiflik oranı ikinci trimester ile kıyaslandığında anlamlı derecede yüksekti sırasıyla %50 ve %15,3 (p<0,05). İzole grupta 2 (%4,8) hastada kromozomal anomali tespit edildi. Bir (%2,4) hastaya trizomi 21 nedeniyle terminasyon uygulandı. Non-izole grupta 13 (%13,4) hastada kromozom anomalisi saptandı ve terminasyon önerildi. Ayrıca 18 (%18,5) hastaya yaşamla bağdaşmayan anomaliler nedeniyle gebelik termiansyonu önerildi. Postnatal değerlendirmede izole ve non-izole grupta cerrahi tedavi oranı sırası ile %6 ve %39,7 idi ve aradaki fark istatiksel olarak anlamlıydı (p<0,05).

PRECIS: We aimed to evaluate the perinatal and neonatal results of pregnant women who were found to have isolated PEV during the first and second trimester ultrasonographic screening in our clinic.

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Sonuç: PEV teşhisi konduğunda, eşlik eden anomaliler için ayrıntılı bir fetal anomali taraması yapılmalı ve hastalar artan kromozomal anomali riski hakkında bilgilendirilmelidir. Tanı için ilk trimesterdeki yüksek yanlış pozitiflik oranları akılda tutulmalıdır. İzole olgulara da karyotip analizi önerilmelidir. Bazı nöromüsküler ve iskelet sistemi anomalilerinin, izole olgularda ilk kez doğum sonrası dönemde ortaya çıkabileceği hatırlanmalıdır.
Anahtar Kelimeler: Çarpık ayak, Down sendromu, tanısal görüntüleme, karyotipleme

Introduction

Congenital pes equinovarus (PEV) or clubfoot is a congenital malformation characterized by excessive plantar flexion of one foot or both feet, inward tilting of the heel, and adduction of the forefoot[1]. It is seen with a frequency of 2-3 per 1000 live births and is the most common congenital deformity of the foot[2]. It can be unilateral or bilateral, and its incidence in male fetuses is 2 times higher[3].

Although the diagnosis of congenital PEV is usually made from the second trimester with ultrasonography (Figure 1), it is possible to diagnose it from 12th week in the late first trimester[4]. Congenital PEV can be diagnosed more frequently through the development of ultrasound technology and the increase in the skills of physicians over time.

Syndromic cases tend to be more complex, and their association with other congenital malformations and/or chromosomal and genetic anomalies is common. It has been associated with some aneuploidies, deletion syndromes, sex chromosome abnormalities, neuromuscular disorders, microdeletion and duplications. Despite advances in molecular gene studies, a major causative gene has not been identified and clinical features of even familial cases show heterogeneity[5].

Positional PEV is mostly associated with intrauterine factors limiting fetal movements, such as oligohydramnios, twin pregnancy and uterine anomalies. Early amniocentesis is one of the iatrogenic reasons that may lead to this[6]. There may be accompanying anomalies or it can be seen as isolated. Idiopathic PEV is usually isolated and generally have a good prognosis, their relationship with chromosomal anomalies is limited, and familial cases have been reported[5]. The fact that it has a higher prevalence in some populations and that it is more common among the male sex suggests that it is the result of a polygenetic predisposition[4].

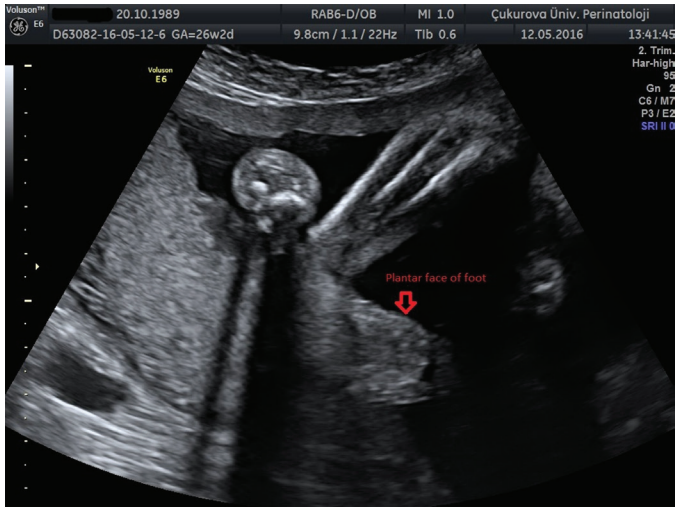

Figure 1. PEV ultrasonography image
PEV: Pes equinovarus

Environmental and genetic factors are thought to play a role in the etiology. It has been shown that maternal smoking, increased body mass index (BMI) and selective serotonin reuptake inhibitor use in the first trimester increases the risk of congenital PEV[7-9]. It was reported that Lamotrigine exposure during the prenatal period increased the risk of congenital PEV[10].

Conservative treatment is the primary method recommended in PEV treatment. Surgery is recommended for patients who do not respond to conservative treatment or who are late for treatment. Untreated patients may experience limitation of motion, deformity, and pain in the long term. Conservative methods include corrective splints (Ponseti or Kite's method), physiotherapy, and serial manipulations[11,12]. The surgical method may vary depending on the severity of the clinical course and accompanying anomalies[13].

In this study, we aimed to evaluate the perinatal, neonatal and orthopedic results of pregnant women who were found to have isolated PEV during the first and second-trimester ultrasonographic screening in our clinic.

Materials and Methods

This study was a retrospective cohort study conducted in the Perinatology Unit of Çukurova University Faculty of Medicine, Department of Obstetrics and Gynecology, between March 2015 and March 2020. Patients who presented to our clinic for routine first-trimester and second-trimester fetal anomaly screening and patients referred to our clinic due to any suspected fetal anomaly or positive screening test were included in this study. Among the patients admitted in the first trimester, those between 12 and 14 weeks were included in the study. All patients were subjected to a detailed fetal anomaly scan and checked for the presence of PEV. Sonographic examinations of all patients were performed by four researchers experienced in fetal anomaly screening using a transabdominal 4-8-MHz probe with a Voluson E6 (GE Medical Systems, Zipf, Austria) device, and information of all patients was recorded on the Microsoft Viewpoint (GE Medical Systems) data recording system. Information of the patients and accompanying anomalies were obtained retrospectively from patient files and Microsoft Viewpoint (GE Medical Systems) program. This study comprised a heterogeneous patient group including high and

low-risk patients. Approval for the study was obtained from the Ethics Committee of Çukurova University Medical Faculty (Registration number: 02.02.2018-74-1). Written informed consent was obtained from all patients.

When PEV was detected, fetal karyotyping was recommended to all patients by performing amniocentesis. Termination of pregnancy was recommended for patients that were detected to have karyotype anomalies and/or severe life-threatening anomalies. Patients whose pregnancy was terminated were not included in the postnatal evaluation. All patients were examined by a pediatrician and an orthopedist after birth. Patients who had no PEV in the neonatal examination were considered as false-positive and they were excluded from the postnatal evaluation. In patients with suspected findings of chromosomal anomalies in postnatal examinations, this was confirmed by karyotype analysis in peripheral blood. In the absence of suspected findings, the karyotype was accepted as normal.

Statistical Analysis

All information including the gestational week at the time of diagnosis, maternal age, BMI, accompanying anomalies, karyotype results, birth week, mode of delivery, and post-natal results were recorded and analyzed. Statistical analysis was performed using the Microsoft SPSS Excel program. The chi-square test was used for comparisons. P-values of <0.05 were considered statistically significant.

Results

During the study period, 7680 patients who presented for routine first and second-trimester fetal ultrasonography screening or who were referred to our clinic due to any suspected fetal anomalies or positive screening tests were examined. One hundred thirty-eight patients with congenital PEV were included in the study. In 41 (29.7%) patients, no accompanying fetal anomalies were observed. In 97 (70.3%) patients, there were some additional fetal anomalies together with PEV. The majority of the patients were diagnosed in the second trimester [128/138 (92.7%)]. Patients diagnosed in the late first trimester (12-13 weeks and 6 days) constituted 7.3% (10/138). Sixty-four (65.9%) patients in the non-isolated group agreed to undergo amniocentesis and chromosomal anomalies were detected in 13 patients. Termination of pregnancy was recommended for 31 patients in the non-isolated group due to severe anomalies. The distribution of these severe anomalies in the non-isolated group was as follows: trisomy 18 in nine patients, trisomy 21 in three patients, trisomy 13 in one patient, neural tube defect in 11 patients, lethal skeletal dysplasia in four patients, and major cardiac anomalies in three patients. Of the 31 patients for whom termination was recommended, 29 accepted the termination. The pregnancy of the remaining 68 patients was followed-up until delivery. Nineteen (46.3%) patients in the isolated group agreed to undergo amniocentesis and chromosomal anomalies were detected in two patients (trisomy 21 in one patient, 47 XXY in one patient). Pregnancy termination was performed to one (2.4%) patient in the isolated group due to trisomy 21.

PEV was unilateral in 58 (42%) patients and bilateral in 80 (58%) patients. The rate of unilaterality was 41.4% (17/41) in the isolated group and was 42.2% (41/97) in the non-isolated group, the difference was not statistically significant (p<0.05). The clinical course of patients diagnosed as having PEV in the prenatal period is summarized in Figure 2.

In the postpartum evaluations (examination and autopsy) performed to confirm the diagnosis of PEV, it was found that some fetuses did not have PEV. When the isolated group and the non-isolated group were compared for false positivity, the false-positive rate was significantly higher in the isolated group (Table 1). The rate of false positivity in the isolated group was found as 17% (7/41). When the isolated group was divided into unilateral and bilateral subgroups and examined, false-positive rates were similar. In the non-isolated group, the rate of false-positives was 5.1% (5/97), and the rate in the unilateral and bilateral subgroups was 9.7% (4/41) and 1.7% (1/56), respectively. The difference between the percentages was not statistically significant (p>0.05).

When the false-positive rates were evaluated according to the week of gestation at the time of diagnosis, the false-positive rates were detected to be significantly higher in the first trimester (Table 2). False positivity was detected in three (30%) of 10 patients diagnosed as having PEV in the first trimester, and nine (7%) of 128 patients diagnosed at the second trimester were found to be false positives. The false-positive rate in the isolated group was up to 50% in the first trimester (Table 2).

When the sex distribution was examined after removing the false-positive cases, the percentage of male fetuses in the isolated and non-isolated groups was 64.7% (22/34) and 66.3% (61/92), respectively, similar to each other and almost twice higher than that of female fetuses.

When compared for characteristic features, no significant difference was found between isolated and non-isolated groups in terms of age, BMI, conception type, unilaterality/bilaterality, mode of delivery, and sex distribution. The clinical features of patients with PEV diagnosed during the prenatal period are shown in Table 3. The mean gestational week at the time of diagnosis, the mean birth week, and mean birth weight was significantly lower in the non-isolated group (Table 3). The rate of pregnancy termination was 2.9% in the isolated group, whereas it was 31.5% in the non-isolated group, the difference was statistically significant (p<0.05). The rate of need for intensive care in the neonatal period was 10% in the isolated group, whereas it was 44% in the non-isolated group, the difference was statistically significant (p<0.05). In the neonatal examination, suspicious findings for chromosomal anomalies were found in two patients in the non-isolated group, and they

were confirmed by karyotype analysis in peripheral blood. Trisomy 21 was detected in one patient and 47 XXY in one patient.

When the frequency of chromosomal anomalies was evaluated according to unilaterality or bilaterality, it was found as 5.88% (3/51) in the unilateral group and 20% (15/75) in the bilateral group, the difference was statistically significant ($p<0.05$).

Trisomy 21 was detected in 51 patients in the entire study group [0.66% (51/7680)]. In five of 51 fetuses with trisomy 21, 9.8% (5/51) had PEV. After the elimination of patients with trisomy

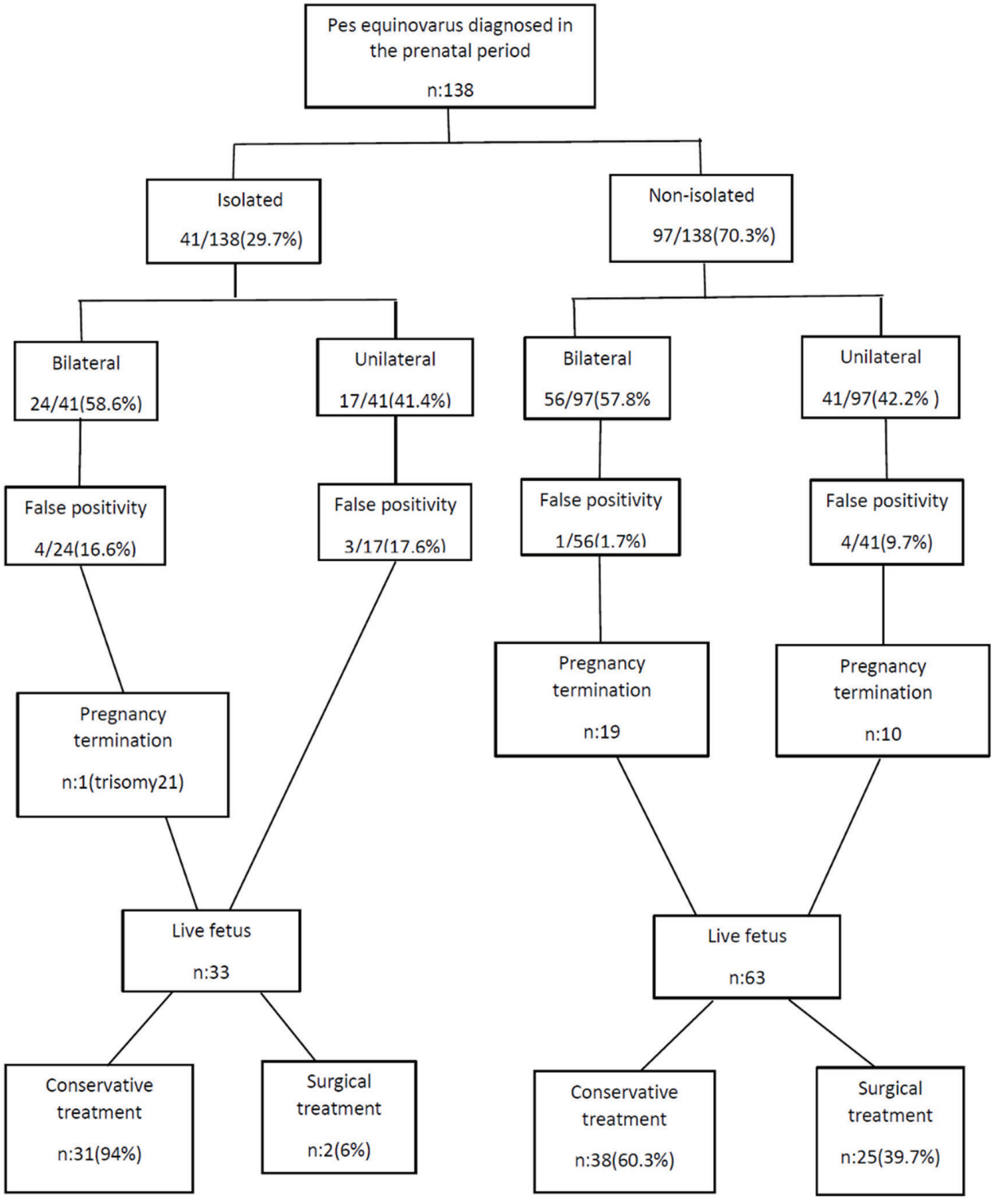

Figure 2. Clinical course of PEV cases diagnosed in the prenatal period
PEV: Pes equinovarus

Table 1. Distribution of postnatal false positivity rate of PEV due to laterality in antenatally diagnosed isolated and non-isolated groups

	Unilateral	Bilateral	Total
Isolated (n=41)	3/17 (16.6%)	4/24 (17.6%)	7/41 (17%)
Non-isolated (n=97)	4/41 (9.7%)	1/56 (1.7%)	5/97 (5.1%)
P-value*			<0.05

*chi-square test was used, PEV: Pes equinovarus

Table 2. Postnatal false positivity distribution according to the gestational week at the time of antenatal diagnosis in the isolated and non-isolated groups

	First Trimester	Second Trimester	p-value*
Isolated (n=41)	1/2 (50%)	6/39 (15.3%)	<0.05
Non-isolated (n=97)	2/8 (25%)	3/89 (3.3%)	<0.05
Total (n=138)	3/10 (30%)	9/128 (7%)	<0.05

*chi-square test was used

21 in the entire group, the PEV rate was calculated as 1.58% (121/7639). The rate of trisomy 21 in patients with PEV was 3.96% (5/126). However, the rate of trisomy 21 in the group without PEV was found as 0.6% (46/7554). This indicates a 6.6-fold increase in the risk for trisomy 21 in patients with PEV. Thirty-one (94%) patients in the isolated PEV group responded to conservative treatment. Conservative treatment was performed on 38 (60.3%) patients in the non-isolated group; the difference was statistically significant (p<0.05). The conservative treatment rate was statistically significantly higher in the isolated group (p<0.05). Surgical treatment was performed on patients who did not respond to conservative treatment or who were late for treatment. Corrective casting (Ponseti) and physiotherapy were used in conservative treatment. In terms of surgery, the most preferred methods were achillotomy and posteromedial release surgery.

Discussion

First-trimester and second-trimester fetal anomaly screening are recommended to all pregnant women during antenatal follow-up. Although PEV incidence is stated as 0.2-0.3% in the literature, the results in our study were far from these values, as some of the patients were referred. Although the diagnosis of PEV is usually made in the second trimester, Keret et al.[4] showed that it was possible to make a diagnosis from the 12th week in the first trimester. However, the diagnosis made in the first trimester has some drawbacks. Bogers et al.[14] showed that there was a temporary PEV position during the normal development of the lower extremity in the first trimester and this development continued until the 13th week. Diagnoses made without waiting for this physiologic positional change will cause an increase in false positivity. In our study, the rate of false positivity in the entire group was 12/138 (8.69%). The false-positive rate was statistically significantly higher in patients in the first trimester than in the second trimester (30% vs. 7%) (p<0.05). In the isolated group, the false-positive rate was found as 7/41 (17%), reaching 50% in the first trimester. In the literature, this rate varies between 0% and 40% for isolated cases[15-18]. Our findings were similar to the literature. However, in the isolated group, 50% false positivity in patients diagnosed in the first trimester draws attention, which can be explained by the fact that the patients in the isolated group were milder and by the physiologic position change of the foot in the first trimester. Due to high false-positive rates, isolated cases,

Table 3. Clinical features of PEV patients diagnosed in the prenatal period

	Isolated n=34	Non-isolated n=92	p-value*
Age	25.32±4.25	26.12±4.72	0.193
BMI	28.3 (21.0-33.0)	25.9 (18.0-31.2)	0.396
Conception method			
Natural IVF-ICSI-ET Other ART	30 (88.2%) 3 (8.8%) 1 (3%)	82 (90.2%) 7 (7.6%) 2 (2.2%)	0.406 0.305 0.105
Gestational week at the time of diagnosis (week)	21.5 (13.0-27.0)	19.1 (13.0-25.0)	<0.05
Laterality			
Unilateral Bilateral	14 (41%) 20 (58.9%)	37 (40.2%) 55 (59. 8%)	0.354 0.413
Fetal Karyotyping Normal Trisomy 21 Trisomy 18 Trisomy 13 Sex chromosomal anomalies	19 (55%) 17 (89%) 1 (5.5%) - - 1 (5.5%)	64 (69%) 51 (79.3%) 3 (4.6%) 9 (14. 6%) 1 (1.5%) -	 0.82 0.79 0.73
Pregnancy result			
Pregnancy termination Delivery Vaginal birth Caesarean	1 (2.9%) 33 (97.1%) 15 (45.5%) 18 (54.5%)	29 (31.5%) 63 (68.5%) 26 (41.2%) 37 (58.8%)	<0.05 <0.05 0.31 0.42
Birth week	38.2 (35.0-39.5)	35.0 (24.6-39.3)	<0.05
Birth weight	3230 (1610-3850)	1715 (595-3770)	<0.05
Sex			
Girl Boy	12 (35.3%) 22 (64.7)	31 (33.7) 61 (66.3%)	0.23 0.24
Neonatal intensive care need	3 (10%)	28 (44%)	<0.05

*chi-square test was used, PEV: Pes equinovarus, BMI: Body mass index

especially those diagnosed in the first trimester, should be followed up with serial examinations to confirm the diagnosis.

In this study, the median gestational week at the time of diagnosis of PEV was determined as 21.5 and 19.1 for the isolated and non-isolated groups, respectively. This can be explained by the fact that the disease is more complex and severe in the presence of accompanying anomalies and therefore can be diagnosed earlier[15,19,20]. Hartge et al.[21] reported the median gestational week at the time of diagnosis as 23 weeks.

Postnatal examinations allow the detection of accompanying findings that could not be found in the prenatal period. Hence, in the non-isolated group, chromosomal anomalies were found in two patients who did not undergo amniocentesis during the antenatal period (trisomy 21 in one patient, 47 XXY in one patient). In the isolated group, no additional anomalies and chromosomal anomalies were found in the postnatal period. Lauson et al.[22] stated that neurologic, developmental, and additional structural anomalies could be detected in postnatal follow-up in isolated cases. In their studies, it was observed that 10% of isolated cases turned into complex cases after a minimum of 1-year follow-up. Di Mascio et al.[20] reported that anomalies related to the skeletal system and neuromuscular system were detected at a rate of 7% in the postnatal follow-up of patients diagnosed as having isolated PEV in the prenatal period. Offerdal et al.[15] made a minimum of 2-years follow-up and found that 15% of the cases turned into complex cases. Shipp and Benacerraf[16] found higher rates in their study. Our study has limitations in this regard because we have not yet followed-up all patients for at least one year.

Our sex distribution (boy/girl) in isolated PEV cases was 2:1, which was consistent with the literature. In our study, the percentage of non-isolated PEV was 73%. Although this rate varies between 48% and 51% in some community studies, rates up to 80% have been reported in tertiary centers such as our clinic where high-risk patients are treated[15,17,23].

In our study, although chromosome anomalies were detected with a rate of 5.8% in the isolated group, the rate was 16.3% in the non-isolated group. Many authors believe that karyotype is necessary in the presence of anomalies accompanying PEV; however, there are ongoing discussions for isolated cases[24,25]. The most common chromosomal anomalies associated with PEV are trisomies 13 and 18. It is possible to recognize cases with trisomy 13 and 18 using ultrasonography due to accompanying anomalies. However, sex chromosome anomalies and trisomy 21 sometimes display very limited or no findings. For this reason, especially in isolated PEV cases, karyotyping comes to the fore due to the risk of trisomy 21. In the study of Viaris et al.[26], the rate of chromosomal anomalies in the isolated group was 2.2%. Lauson et al.[22] reported the incidence of chromosomal anomalies as 2.3% in the isolated group. Recommending karyotype to isolated cases in the presence of advanced maternal age or positive screening test should not be avoided[20]. PEV was detected in five of 51 patients with trisomy 21 in our study population (9.8%). This situation increases the risk of trisomy 21 in patients with PEV 6.6 times. Offerdal et al.[15] detected aneuploidy at the rate of 13% in patients with PEV and showed that there was a strong relationship between PEV and chromosomal anomalies, and they recommended karyotyping for all patients including isolated cases. Tegnanader and Eik-Nes[27] reached similar results.

According to the findings of Bakalis et al.[17], the prognosis was worse and the frequency of chromosomal anomalies was higher in the bilateral group, similar to our study. In the study of Viaris et al.[26], no difference was found between the two groups.

Despite the high incidence of PEV, only a few causative genes are known. *PITX1 (MIM 602149), IGFBP3 (MIM 146732), TBX4*, and ***RBM10*** genes have been found to be associated with isolated PEV[28-30]. Recommending chromosomal microarray studies in addition to conventional karyotyping to patients with PEV will help to better understand the factors causing the disease because multigenetic factors play a role in the etiology[20].

Conservative treatment is the primary method recommended in the treatment of PEV. Surgery is recommended for patients who do not respond to conservative treatment. There is no treatment in the prenatal period. The accepted method in conservative treatment is Ponseti casting (12). In our cases, surgical treatment was performed on two patients (6%) in the isolated group and 25 (39.8%) patients in the non-isolated group, the difference was statistically significant ($p<0.05$). Careful fetal anomaly screening performed to detect accompanying anomalies will help us understand whether the cases are isolated and predict the postnatal prognosis.

Study Limitations

The limitations of this study are that it had a retrospective design, only patients diagnosed during the intrauterine period could be reached, and the rate of PEV detection could not be specified due to the exclusion of patients diagnosed for the first time in the postnatal period.

Another limitation is that only conventional karyotyping could be performed in patients, chromosomal microarray was not performed. Studies involving chromosomal microarray evaluations are needed to better understand the etiology of the disease.

Conclusion

The diagnosis of PEV can be made in the late first and second trimesters. In the first trimester, the rate of false positivity is higher, and the diagnosis should be confirmed with serial examinations. When PEV is diagnosed, detailed fetal anomaly screening should be performed for anomalies that may accompany, and patients should be informed about the

increased incidence of chromosomal anomalies, and karyotype and chromosomal microarray analysis should be recommended. Chromosomal microarray can identify clinically significant chromosome abnormalities (gains and losses of DNA) that are below the resolution of conventional chromosome analysis. The risk of this chromosomal and structural anomaly further increases in the presence of accompanying additional findings. It should be kept in mind that some neuromuscular and skeletal system anomalies may occur for the first time in the postnatal period in isolated cases.

Ethics

Ethics Committee Approval: Approval for the study was obtained from the Ethics Committee of Çukurova University Medical Faculty (Registration number: 02.02.2018-74-1).

Informed Consent: Written informed consent was obtained from all patients.

Authorship Contributions

Surgical and Medical Practices: S.C.D., Concept: M.S., Design: M.S., Data Collection or Processing: M.S., Analysis or Interpretation: S.C.D., Literature Search: S.C.D., Writing: S.C.D.

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

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A prospective study of a new prediction model of vaginal birth after cesarean section at a tertiary care centre

Üçüncü basamak bir merkezde sezaryen sonrası vajinal doğum için yeni bir tahmin modelinin prospektif çalışması

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Abstract

Objective: To create a new and simple model for predicting the likelihood of vaginal birth after cesarean (VBAC) section using variables available at the time of admission.

Materials and Methods: A prospective observational study was performed at a tertiary care centre in Haryana over a period of 12 months (January 2018 - December 2018) in pregnant women attending the labour room with one previous cesarean section fulfilling the criteria for undergoing trial of labour after cesarean (TOLAC). The sample size was 150. A VBAC score was calculated for each patient using a new prediction model that included variables available at the time of admission such as maternal age, gestational age, Bishop's score, body mass index, indication for primary cesarean section, and clinically estimated fetal weight. The results of the VBAC scores were correlated with outcomes i.e. successful VBAC or failed VBAC. The chi-square test and Student's t-test was used for comparison among the groups. Descriptive and regression analysis was performed for the study variables.

Results: Out of 150 TOLAC cases, 78% had successful VBAC and the remainder (22%) had failed VBAC. The observed probability of having a successful VBAC for a VBAC score of 0-3 was 34%, 4-6 was 68%, 7-9 was 90%, and ≥10 was 97%. The prediction model performed well with an area under the curve of 0.77 (95% CI: 0.68 to 0.85) of the receiver operating characteristics receiver operating characteristic curve.

Conclusion: The present study shows that the proposed VBAC prediction model is a good tool to predict the outcome of TOLAC and can be used to counsel women regarding the mode of delivery in the current and subsequent pregnancies. Further studies of this model and other such models with different permutations and combinations of variables are required.

Keywords: Vaginal birth after a cesarean, prediction, model

Öz

Amaç: Bu çalışma, başvuruda olan değişkenleri kullanarak sezaryen sonrası vajinal doğum (SSVD) olasılığını tahmin etmek için yeni ve basit bir model oluşturmak için yapılmıştır.

Gereç ve Yöntemler: Haryana'daki üçüncü basamak sağlık merkezinde 12 aylık bir süre boyunca (Ocak 2018 - Aralık 2018) doğumhaneye başvuran, daha önce bir kez sezaryen ile doğum yapan ve "Gebelerde Sezaryen Sonrası Doğum Çalışması" (TOLAC) kriterlerini karşılayan gebelerde prospektif bir gözlemsel çalışma yapıldı ve örneklem büyüklüğü 150 idi. Her hasta için SSVD skoru, maternal yaş, gebelik yaşı, Bishop skoru, vücut kitle indeksi, birincil sezaryen endikasyonu ve klinik olarak tahmin edilen fetal ağırlık gibi hastaneye başvurudaki değişkenleri içeren yeni bir tahmin modeli kullanılarak hesaplandı. SSVD skorunun sonuçları, başarılı veya başarısız SSVD şeklinde sonlanım ile ilişkilendirildi. Gruplar arası karşılaştırmada ki-kare testi ve Student's t-testi kullanıldı. Çalışma değişkenleri için tanımlayıcı analiz ve regresyon analizi yapılmıştır.

Bulgular: Yüz elli TOLAC kriterlerine uyan gebenin %78'inde başarılı SSVD ve %22'sinde başarısız SSVD gözlendi. SSVD skoru 0-3 için başarılı bir SSVD'ye sahip olma olasılığı %34, 4-6 için 68, 7-9 için %90 ve >10 için %97 idi. Tahmin modeli, alıcı çalışma karakteristikleri analizinde; 0,77'lik (%95 güven aralığı 0,68 ila 0,85) eğri altındaki alan ile iyi performans göstermiştir.

Sonuç: Bu çalışma, önerilen SSVD tahmin modelinin TOLAC sonucunu tahmin etmek için iyi bir araç olduğunu ve kadınlara mevcut ve sonraki gebeliklerde doğum şekli konusunda danışmanlık yapmak için kullanılabileceğini göstermektedir. Bu modelle ve farklı permütasyonlara ve değişken kombinasyonlarına sahip diğer bu tür modellerle ilgili daha fazla çalışma yapılması gerekmektedir.

Anahtar Kelimeler: Sezaryen sonrası vajinal doğum, tahmin, model

PRECIS: A new prediction model of vaginal birth after cesarean containing factors available at the time of admission was tested and it was found to be a good tool.

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Introduction

The effective and safe use of cesarean delivery has been a focus and concern for last the three decades. It was the result of the 1980 National Institutes of Health Consensus Conference on Cesarean Childbirth held in response to the three-fold increase in the rate of cesarean deliveries (from 5% in 1970 to 15.2% in 1978) that vaginal birth after cesarean (VBAC) came into being[1]. As a result, the VBAC rate rose from 19.9% in 1990 to 28.3% in about a decade and the cesarean delivery rate decreased from 22.7% to 20.7%[2]. Later, with increasing incidence of uterine rupture, VBAC had a setback and went into disrepute, once again leading to an increase in the cesarean rates. This has led to significant research in determining the best permutations and combinations of the factors to achieve the optimum outcome of a previous cesarean delivery. There are numerous factors such as maternal age, body mass index (BMI), gestational age, spontaneous or induced labor, inter-conception period, estimated fetal weight, Bishop's score, type of previous cesarean scar, and indication for primary cesarean delivery, which can influence the decision to undergo a trial of labour after cesarean (TOLAC) and its outcome i.e. failed VBAC (emergency repeat cesarean section) or successful VBAC (vaginal delivery).

Rates of maternal complications are highest among women who attempt vaginal birth and fail, intermediate among women who have planned cesarean delivery, and lowest among women who attempt vaginal birth and succeed[3]. VBAC success rates also vary between institutions and service providers. Thus, it is worth remarking that as of now, there is no reliable and demonstrable algorithm or nomogram that correctly identifies or accurately predicts the success of VBAC[4]. Hence, management of a case of previous lower segment cesarean section continues to be an obstetric dilemma.

Therefore, an accurate and reliable prediction model must be designed and validated to predict a successful outcome, but literature is scarce from India that could assess the ante-partum and intrapartum determinants for predicting successful VBAC. Hence, this study was planned. The aim of the study was to create a new model for predicting the likelihood of VBAC using variables available at the time of admission.

The objective of the study was to test the performance of the prediction model for success of VBAC delivery.

Materials and Methods

This prospective observational study was conducted at a tertiary care centre over a period of 12 months (January 2018 to December 2018) in pregnant women attending the labour room with one previous cesarean section. This hospital a referral center for three major districts of Haryana State in North India with annual live birth rates ranging from 4,500 to 5,000, average overall cesarean rate of 20-25% of total deliveries, and a repeat cesarean rate of 30-35% of total cesareans. At 5% alpha error, 80% power and 95% confidence interval (CI), the sample size calculated using Master 2.0 software (India) was 150. Ethical committee approval of the study was obtained (approval number: BPSGMCW/RC279/IEC/18) and informed and written consent was given by each patient who fulfilled the following inclusion and exclusion criteria to undergo TOLAC.

Inclusion criteria: Singleton pregnancy, vertex presentation, one prior LSCS with non-recurrent indication, gestational age ≥37 weeks confirmed in first-trimester scan and menstrual history, maternal age (18-35 years).

Exclusion criteria: Age <18 years or >35 years, intrauterine fetal death, lethal fetal anomalies, non-reassuring fetal heart rate on admission, cephalopelvic disproportion, malpresentation, history of antepartum haemorrhage or adherent placenta in the current pregnancy, history of uterine surgery other than cesarean section.

The following system was designed using the relative weights of significant factors used in previous models given by Troyer and Parisi et al.[5], Flamm and Geiger[6], Grobman et al.[7,8], Wen et al.[9], and Metz et al.[10]. In the proposed model, we included six variables, four of which, namely maternal age in years, gestational age in weeks, indication for primary cesarean, and BMI were also included in Grobman's model. In place of cervical dilation, we used Bishop's score, which was the main factor in the study by Metz et al.[10] Estimated fetal weight was the sixth variable, which was also used in the study by Wen et al.[9,10] There are only a few Indian studies on prediction models and most studied individual factors instead of prediction models, which is why statistically significant factors previously studied in Indian studies as well as supported by the American College of Obstetrics and Gynecology and the Royal College of Obstetrics Gynaecology guidelines were included[3,11-13]. Each variable used in the prediction model was assigned a score of 0, 1, or 2. Scores were decided based on the previous models e.g Flamm and Geiger[6] and Troyer and Parisi et al.[5] who gave a score of 0 to those with a primary indication of cesarean section as failure to progress[12,14]. A score of 2 was chosen for breech and fetal distress because, according to the literature, this group had a statistically significant favourable VBAC outcomes in TOLAC studies. Similarly, other factors were given scores accordingly. A pilot study of this model was performed on 50 patients, the results were analyzed, necessary corrections were made, and later it was performed on 150 more patients.

VBAC scoring system used in the proposed prediction model:

1. Maternal age (in years): a. >30=0 b. 25-30=1c.18-25=2
2. Gestational age (in weeks): a. <39=0 b. 39-40=1 c. >40=2
3. Indication for primary caesarean-section:

a. Non-progress of labor (NPOL) and others = 0

b. Intrauterine growth restriction (IUGR), oligohydramnios, Antepartum haemorrhage =1

c. breech presentation or fetal distress =2

4. Bishop score: a. 0-3=0 b. 4-5=1 c. 6-10=2

5. BMI in kg/m^2 on admission:
a. 30=0 b. 25-29=1 c. <25=2
6. Clinically estimated fetal weight in grams according to Johnson's formula:
a. >3500=0 b. 2500-3500=1c. <2500=2
VBAC scores were calculated for each patient fulfilling the criteria to undergo TOLAC at the time of admission and the result obtained was correlated with the outcome i.e. failed VBAC or successful VBAC.

Statistical Analysis

Statistical package for the social sciences version 20 was used for statistical analysis. Descriptive statistics were used for the demographic features such as age, parity, gestational age, BMI, Bishop's score, and the indication for primary cesarean section. The chi-square test and Student's t-test was used for comparisons among the groups. Multivariate logistic regression analysis using the enter method was performed to calculate the adjusted odds ratio for each factor used in the VBAC prediction model to determine their association with successful VBAC. A p-value of ≤0.05 was considered statistically significant. Finally, the receiver operating characteristic (ROC) curve was measured by calculating the corresponding area under the curve (AUC) and 95% CI.

Results

Out of 150 TOLAC cases, 78% had successful VBAC and the remainder (22%) had failed VBAC (emergency repeat cesarean section). Table 1 depicts the individual variables of the VBAC prediction model, their frequency distribution, and their means with standard deviation. Table 2 shows the indications of cesarean section in the failed VBAC group. The most common indication was fetal distress followed by scar tenderness and failed induction in 27% (9/33), 25% (8/33), and 21% (7/33), respectively. Out of eight cases of scar tenderness, three had thinned out scar of previous cesarean section.

We developed a total score of 0-12. The final cumulative VBAC score ranged from 2 to 11 in the present study. It was 10 or more in 5.30%, 7 to 9 in 47.3%, 4 to 6 in 40.10%, and 3 or less in 7.30% of the cases. As can be seen in graph 1, the observed probability of having a successful VBAC for VBAC score 0-3 was 34%, 4-6 was 68%, 7-9 was 90%, and ≥10 was 97%. The predicted VBAC percentages also mentioned in graph 1 were calculated using binary logistic regression analysis and were closely related to the observed ones.

The multivariate regression analysis of all six variables as depicted in the Table 3 shows that two variables i.e. gestational age and Bishops score had the odds of 2.047 and 3.082,

Table 1. Demographic characteristics of women undergoing trial of labour (*chi-square and Student's t-test as appropriate)

Demographics characteristics	Failed VBAC % (n/N)	Successful VBAC % (n/N)	p
Number of women underwent VBAC (150)	22 (33/150)	78 (117/150)	
Maternal age (yrs) <25 25-30 >30	25.93±3.51 48.49 (16/33) 39.39 (13/33) 12.12 (04/33)	25.82±3.70 52.14 (61/117) 35.90 (42/117) 11.96 (14/117)	0.92
BMI (kg/m^2) <25 25-30 >30	25.08±3.62 63.64 (21/33) 24.24 (8/33) 12.12 (4/33)	23.19±2.63 79.49 (93/117) 17.95 (21/117) 2.56 (03/117)	0.04
Gestational age (weeks) <39 39-40 >40	38.12±1.36 78.79 (26/33) 18.18 (6/33) 3.03 (1/33)	38.96±1.24 62.39 (73/117) 26.49 (31/117) 11.12 (13/117)	0.16
Indication of primary cesarean NPOL & others IUGR, oligohydramnios, APH breech, fetal distress	 54.55 (18/33) 9.09 (3/33) 36.36 (12/33)	 23.08 (27/117) 17.09 (20/117) 59.83 (70/117)	<0.01
Clinically estimated fetal weight (grams) <2500 2500-3500 >3500	3389.54±471.45 0 6.06 (2/33) 45.46 (15/33) 48.48 (16/33)	3402±470.5 02.57 (3/117) 46.15 (54/117) 51.28 (60/117)	0.61
Bishop's score 0-3 4-5 6-10	3.60±0.82 48.48 (16/33) 51.52 (17/33) 0.0 (0/33)	6.34±2.35 11.97 (14/117) 25.64 (30/117) 62.39 (73/117)	<0.01

Data are expressed as percentages with frequencies in the parentheses and mean ± standard deviation, VBAC: Vaginal birth after cesarean, BMI: Body mass index, NPOL: Non-progress of labour, IUGR: Intrauterine growth restriction, APH: Antepartum haemorrhage

Table 2. Indication of cesarean section in failed VBAC group

Indication of cesarean in failed VBAC group	% (n/N)
Fetal distress	30.03 (10/33)
Scar tenderness	27.27 (09/33)
Failure of induction of labour	24.24 (08/33)
Non-progress of labour	18.18 (06/33)
Total	100.00 (33)

VBAC: Vaginal birth after cesarean

Table 3. Multivariate regression analysis of the variables included in the prediction model

Characteristics	Beta coefficient	Adjusted odds ratio	p	95% CI
Maternal age in years	-0.148	0.862	0.146	0.706-1.053
Gestational age in weeks	0.716	2.047	0.005	1.244-3.369
Body mass index in kg/m^2	-0.184	0.832	0.042	0.696-0.994
Bishop's score (0-13)	1.126	3.082	<0.001	1.882-5.048
Estimated fetal weight in grams	0.000	1	0.944	0.999-1.001
Indication of primary cesarean[a]				
1	-0.092	0.912	0.908	0.189-4.405
2	-0.542	0.581	0.514	0.114-2.963

[a]1-non-progress of labour (NPOL) and others, 2-IUGR, oligohydramnios, Antepartum haemorrhage 3-breech presentation or fetal distress, Constant value -29.749, CI: Confidence interval

Table 4. Multivariate regression analysis of additional variables not included in the prediction model

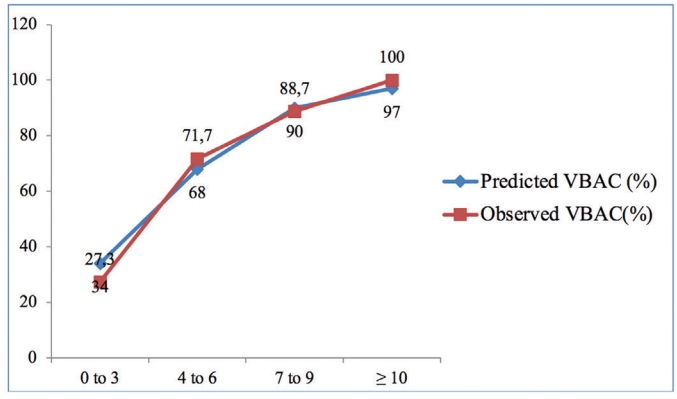
Characteristics	Beta coefficient	Adjusted odds ratio	p	95% CI
Spontaneous onset of labour (yes/no)	0.948	2.580	0.043	1.032-6.450
Interdelivery interval in months	0.041	1.042	0.031	1.004-1.082
Previous NVD (yes/no)	0.667	1.947	0.272	0.592-6.405
Previous VBAC (yes/no)	1.366	3.918	0.210	0.465-33.163
Parity	1.637	5.138	0.010	1.471-17.943

Constant value -29.749, VBAC: Vaginal birth after cesarean, CI: Confidence interval

respectively, for having a successful VBAC with significant p-values in both, and with each 1 unit increase in BMI the odds of having a successful VBAC reduced by 0.832 ($p<0.05$). The other three factors i.e. age, indication for previous cesarean, and estimated fetal weight (Johnson's formula) were not significantly associated with successful VBAC.

As shown in Table 4, we studied five additional variables out of the model variables, namely spontaneous onset of labour, parity, interdelivery interval in months, previous history of successful VBAC, and previous history of normal vaginal delivery. These variables were chosen because they were also included in the previous models by Grobman and Flamm, Geiger and Wen et al.[6,8-10]. Out of these five, two i.e. spontaneous onset of labour and parity were significantly associated with successful VBAC with odds of 2.58 and 5.138, respectively; the remaining three had no significant effect.

Seventeen (11.3%) patients out of 150 who underwent TOLAC had complications such as neonatal intensive care unit admissions (n=6), failed and successful VBAC groups (n=3 for



Graph 1. Predicted compared with observed vaginal birth after cesarean section (VBAC) (Successful TOLAC)

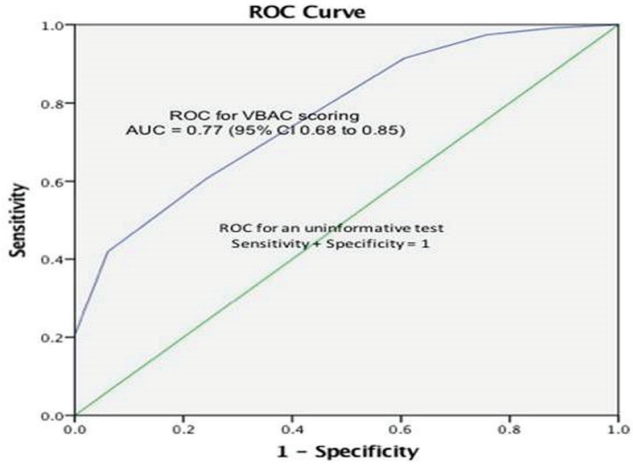


Figure 1. ROC curve for the scoring system showing area under the curve (AUC)

ROC: Receiver operating characteristic curve

both), and bladder injury (n=1) in the failed VBAC group, and a total of 10 cases of PPH (all atonic PPH), four in the successful VBAC group and six in the failed VBAC group. There were no cases of uterine rupture and ICU admissions in the present study.

Figure 1 shows the ROC curve with an AUC equal to 0.77 (95% CI: 0.68 to 0.85).

Discussion

The ACOG 2010 quoted the success rate of VBAC as 60-80%[3]. VBAC success rates also vary between institutions and service providers. The success rate of VBAC in the present study was 78%, similar to the quoted percentage. The mean age of the women in the present study was 25.84±4.20 years and there was no significant difference in the ages of the women in the two groups i.e. successful VBAC and failed VBAC groups, similar to study by Metz et al.[10] (27.9±4.3 and 27.5±4.6; p=0.20). The mean age in the current study was lower as compared with the study by Xing et al. [15] because 76.6% of the women were from rural areas where young marriage and childbirth is common; the mean age in the successful VBAC and failed VBAC group was higher and there was a significant difference between the two groups (27.9±4.8 and 35.5±4.7; p=0.043).

Troyer and Parisi[5] in 1992 studied 264 women with one previous cesarean section and developed a model with four factors, namely previous dysfunctional labour, non-reassuring fetal heart tracing at admission, no previous vaginal delivery, and labour induction. Each variable was given one point and the total score ranged from 0-4. The patients with the lowest score (i.e. 0) had the highest VBAC success rate (91.5%) as compared with those with higher scores. This model has not been studied extensively and needs further research and validation. The indication of primary cesarean section i.e. previous dysfunctional labour was also included in the present study, mentioned here as NPOL, and was similarly assigned a zero score.

There is a popular model known as the Flamm scoring system, which was developed in 1997 in California[6]. The research was performed on 5022 pregnant women including four variables known at the time of admission i.e. age of the patient, vaginal delivery before and after the cesarean section, a non-recurring indication of primary caesarean, cervical dilatation and cervical effacement. The result was given as a score of 0-10 and each score had a different percentage of success i.e. 0-2 corresponded to 49.1%, 3-7 corresponded to 59.9%, 66.7%, 77%, 88.65%, and 92.65%, and the success of 8-10 was 94.9%. In the present study, we used two of the above factors in the VBAC prediction model i.e. patient age, but instead of cervical dilation, we used Bishop's score.

Patel et al.[12] from Gujarat (India) in 2016 performed a prospective observational study on 150 women with one previous cesarean section using the Flamm scoring system. They found a mean Flamm score for successful VBAC of 5.35 (95% CI: 3.9 to 6.7) compared with failed VBAC i.e. 3.62 (95% CI: 27 to 4.57) and the chances of success of TOLAC increased with increasing Flamm scores. Similarly, in present study, the mean VBAC score of the failed VBAC group was 5.03±1.82 and that of the successful VBAC group was 7.01±1.77 (95% CI: 1.28 to 2.67; p<0.001). The authors concluded that Flamm scoring gave a fair judgment of successful vaginal birth in TOLAC, and using Flamm scores and monitoring though partogram would reduce the rate of cesarean sections in patients with one previous lower segment cesarean section. However, this model also has limited supprting data and needs further validation.

The most studied prediction model of VBAC was developed by Grobman of the northwestern University of Chicago in 2007[8]. It included six variables: maternal age, BMI, ethnicity (e.g. African-American/Hispanic), any previous vaginal delivery, any vaginal delivery since the last cesarean, and indication for primary cesarean of the arrest of dilation or descent. All variables were those that could be determined at the first antenatal visit with the idea of starting the counselling in the first trimester. Later, this model was improved in 2009 by adding certain other factors like most recent BMI within 2 weeks of delivery, gestational age at delivery, gestational diabetes mellitus, preeclampsia, cervical examination findings at admission, and the undertaking of labour induction. The result was expressed as the percentage of success of TOLAC. Inclusion of these additional factors slightly improved the performance of the calculator. Similarly, we tried to include those variables in the present prediction model, which were available at the time of admission because our hospital is a referral hospital and most of the time our patients are unscheduled (77.3% in the present study).

The AUC of the ROC curve of Grobman's 2007 model was 0.751, and that of the new model was 0.779, and these values were significantly different (p<0.001)[9]. This model is currently known as the MFMU calculator and is freely available on the internet. The AUC of the ROC curve of our model was 0.77, similar to that of Grobman's 2007 and 2009 models (0.751 and 0.779), which suggests that the proposed prediction model performed well.

In 2018, Wen et al.[9] conducted a retrospective cohort study on 444 women with one cesarean delivery and at least one subsequent attempt for a trial of labor in Nanjing, China. They used Grobman's model and also a modified version of this model and compared the two. The considered potential VBAC predictors included Grobman's background variables and two new variables, maternal height and estimated fetal weight. Their overall VBAC success rate was 83.3%. The AUC of Grobman's model was 0.831 (95% CI: 0.775 to 0.886), and the AUC of their own modified model with two new variables added was 0.857 (sensitivity =72.2%, specificity =83.8%). However, the difference between the AUC of the two models was not significant (Z=-1.69, p=0.091). Hence, they found that Grobman's model was well accepted in the Chinese population, also that the modified model supplemented with maternal

height and estimated fetal weight needed to be further studied in the Chinese population.

There was no case of uterine rupture in the present study, whereas the incidence was 0.90% in the study by Patel et al.[12] and 0.28% in the study by Xing et al.[15] A recent meta-analysis suggested that measurement of lower uterine segment (LUS) thickness antenatally in women with a previous caesarean delivery could be used to predict the occurrence of a uterine defect (scar dehiscence or scar rupture) in women undergoing VBAC[16]. Further prospective observational studies are needed using a standard method of intrapartum LUS thickness measurement to predict the outcome of TOLAC and risk of uterine rupture.

The present study included a very important variable i.e. Bishop's score that has not been incorporated in any of the popular models by Flamm and Geiger[6] and Grobman et al.[8], who included only the individual components of Bishop's score such as cervical dilation, effacement, and station. Metz et al.[10] and Xing et al.[15] included Bishop's score in their model, despite it being a subjective variable, its importance was highlighted in their study also[10,15]. Metz et al. [10]. used its value as the main factor in developing a score to which a value of 2 to 4 was added for another four variables (history of vaginal delivery, BMI, primary cesarean delivery because of nonrecurring indication, maternal age <35 years) to get the final VBAC score. In the present study, it has an adjusted odds ratio of 3.08 and has a very strong association with successful VBAC ($p<0.05$).

Spontaneous onset of labour and parity are two other important variables that may be incorporated in the present model and a further study can be planned. There are insufficient studies about VBAC prediction models and most studied only individual variables. Other variables studied in other prediction models are weight gain in pregnancy, preeclampsia, gestational diabetes, insurance. and race[8,9,15]. There is a need for the development of a standard prediction model and further studies of this model and many more such models with different permutations and combinations of various variables are required to help predict the success of TOLAC with high accuracy.

Study Limitations

The small sample size is the limitation of the present study. The study was approved by the institutional ethics committee and was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. There is no conflict of interest among the authors and this study was not funded by any organization.

Conclusion

The present study tests a new VBAC prediction model and shows that it is a good tool for predicting VBAC and hence can be used to counsel women regarding the mode of delivery in current and subsequent pregnancies. Parity, spontaneous onset of labour, admission Bishop's score, gestational age, and BMI were the factors with a statistically significant association with successful VBAC. Further studies could be proposed such as the comparison of two different types of scoring systems, each system with different variables, in a given population.

Ethics

Ethics Committee Approval: Ethical committee approval of the study was obtained (approval number: BPSGMCW/RC279/IEC/18).

Informed Consent: Informed and written consent was given by each patient

Peer-review: Externally peer-reviewed.

Authorship Contributions:

Surgical and Medical Practices: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M., Concept: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M., Design: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M., Data Collection or Processing: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M., Analysis or Interpretation: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M., Literature Search: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M., Writing: P.L., B.P., S.S., M.U., S.Sh., V.S., R.M.

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Impact of the expanded examination of fetal heart to the prenatal diagnosis of congenital heart diseases

Genişletilmiş fetal kardiyak değerlendirmenin konjenital kalp hastalıklarının prenatal tanısına etkisi

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Abstract

Objective: In the present study, for which reasons fetal cardiac evaluation was requested from our pediatric cardiology clinic, the effects of routine fetal cardiac evaluation in obstetric ultrasonography (USG) on the detection of congenital heart disease (CHD) and the distribution of intrauterine diagnosis of CHD according to pregnancy risk profiles were retrospectively analyzed.

Materials and Methods: Fetal echocardiography reports which containing the nineteen-month period were retrospectively examined. We performed a fetal echocardiography for all pregnant women who were referred to pediatric cardiology clinic after detail obstetric USG screening. The pregnancies were categorized into two groups based on the risk of CHD: Low-risk and high-risk groups. Detected congenital cardiac structural malformations were classified as complex, moderate, and mild according to perinatal mortality risk.

Results: Of the 736 pregnancies, 22 were twin, and fetal cardiac evaluation was performed in 758 fetuses. There were 341 (46.3%) pregnancies in the high-risk group and 395 (53.6%) pregnancies in the low-risk group. The most common reason for fetal cardiac evaluation request was inability to adequately visualize the fetal heart (36.1%), while suspected fetal cardiac abnormality was the second most common cause (21.3%). Number of fetuses detected with cardiac abnormalities was 80 (23.5%) among high-risk pregnancies, and 20 (5%) among low-risk pregnancies. The most common type of malformation was simple cardiac abnormalities (6%) followed by complex lesions (4.1%). The most common cardiac abnormality was ventricular septal defect comprised of 18 cases (2.4%) while the most common complex cardiac abnormality was pulmonary atresia (1.2%). The rate of consistency was 40.1% between obstetricians and pediatric cardiologist in terms of the diagnosis of the congenital cardiac malformations.

Conclusion: Routine evaluation of the fetal heart by means of obstetric USG, including four chambers, outflow tracts' and three vessel views, would allow for diagnosing congenital cardiac malformations to a large extent during the intrauterine period.

Keywords: Congenital heart disease, fetal echocardiography, high risk pregnancy, low risk pregnancy, prenatal diagnosis

Öz

Amaç: Bu çalışmada pediatrik kardiyoloji kliniğimizden fetal kardiyak değerlendirmenin hangi nedenler ile talep edildiği, obstetrik ultrasonografide (USG) rutin fetal kardiyak değerlendirmenin konjenital kalp hastalıklarının (KKH) fetal ekokardiyografi ile tespitine etkisi ve intrauterine tanı koyulan KKH'nin gebelik risk profiline göre dağılımı retrospektif olarak incelenmiştir.

Gereç ve Yöntemler: On dokuz aylık döneme ait fetal ekokardiyografi raporları retrospektif olarak incelendi. Fetal ekokardiyografik değerlendirme, ayrıntılı obstetrik USG taraması sonrasında pediatrik kardiyoloji kliniğine yönlendirilen tüm gebelere yapıldı. Gebeler KKH'li fetusa sahip olma risklerine göre düşük ve yüksek riskli olarak iki gruba ayrıldı. Tespit edilen konjenital kardiyak malformasyonlar perinatal mortalite riskine göre kompleks, orta ve hafif derece olmak üzere sınıflandırıldı.

Bulgular: Toplam 736 gebenin 22 tanesi ikiz gebelik olup, 758 bebeğe fetal kardiyak değerlendirme yapıldı. Yüksek risk grubunda 341 (%46,3), düşük risk grubunda ise 395 (%53,6) gebelik mevcuttu. Fetal ekokardiyografik değerlendirme isteminin en sık nedeni fetal kalbin görüntülenmesinde yetersizlik (%36,1) olup, fetal kardiyak anormallik şüphesi ikinci en sık nedendi (%21,3). Kardiyak anomali saptanan fetus sayısı yüksek riskli gebelerde 80 (%23,5), düşük riskli gebelerde 20 (%5) idi. En yüksek oranda saptanan malformasyon tipi hafif kardiyak anomaliler olup (%6), kompleks lezyon saptanma oranı ikinci sıradaydı (%4,1). En sık saptanan kardiyak anomali ventriküler septal defekt 18 (%2,4) olup, en sık saptanan kompleks kardiyak anomali ise

PRECIS: The expanded examination of the fetal heart by means of obstetric USG increases the rate of prenatal detection of congenital heart defects.

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pulmoner atreziydi (%1,2). KKH'nin tanınması açısından obstetrisyen ve pediatrik kardiyoloji arasındaki uyum oranı %40,1 idi.
Sonuç: Fetal kalbin dört boşluk, ventriküllerin çıkım yolları ve üç damar kesiti rutin kapsayacak şekilde obstetrik USG sırasında değerlendirilmesi, konjenital kardiyak malformasyonların intrauterin dönemde büyük oranda tanı almasına imkan sağlayabilir.
Anahtar Kelimeler: Konjenital kalp hastalıkları, fetal ekokardiyografi, yüksek riskli gebelik, düşük riskli gebelik, prenatal tanı

Introduction

Congenital heart disease (CHD) is the most common congenital abnormality, and it is six times more common than chromosomal abnormalities and four times more common than neural tube defect[1]. The incidence of CHD is 8-10 cases per 1000 live births (0.8%-1%) for full-term births, and this rate is approximately 10 times more (8.3%) for preterm births[2]. Approximately 17%-33% of CHDs include critical malformations, which must be intervened directly after birth and/or just before birth. Unfortunately, 50% of these malformations are diagnosed after the infant is discharged from the hospital, and the mortality risk increases because of the delay. The identification of severe cardiac abnormalities during the intrauterine period enables the use of approaches that lead to significant decreases in perinatal morbidity and mortality, such as performing the delivery at a center where cardiac surgery can be performed, providing the required medical support in the newborn intensive care unit until transfer to the relevant center, and/or if needed, promptly performing transcatheter palliative interventions. Therefore, fetal cardiac evaluation has become an important part of obstetric ultrasonography (USG), and fetal echocardiography evaluation requests have increased due to increased incidences of suspected CHD. Although the risks of CHD are defined and fetal cardiac evaluation is recommended for all high-risk pregnancies, 90% of CHDs are observed in low-risk pregnancies[3,4].

The current approach in many clinics in Turkey involves performing fetal cardiac evaluations within defined indications owing to long examination times and inadequate number of pediatric cardiologists and cardiovascular surgery experts. Our hospital is a tertiary center and includes the only perinatology and pediatric cardiology clinic that provides services to a large region, which consists of our city and neighboring cities. In the present study, for which reasons fetal cardiac evaluation was requested from our pediatric cardiology clinic, the effects of routine fetal cardiac evaluation in obstetric USG on the detection of CHDs and the distribution of intrauterine diagnosis of CHDs according to pregnancy risk profiles were retrospectively analyzed.

Materials and Methods

In this retrospective study, the total number of anomaly scans in the perinatology unit during the study period (May 2017 - January 2019) were 8372 and the number of referred pregnancies for fetal echocardiography evaluations were 736. All patients in the study underwent routine fetal anatomic scanning according to the International Society of Ultrasound in Obstetrics and Gynecology guidelines between the 18th and 22nd weeks of pregnancy in the Department of Perinatology at our university. In this scan, four chambers of the heart, three vessel and trachea, left and right ventricular outflows, and the heart were examined using color Doppler USG[5]. Subsequently, the patients were referred to the Department of Pediatric Cardiology unit form perinatology division of our university hospital either suspected cardiac abnormalities, accompanying diseases that increase risk of fetal cardiac malformations, parental congenital heart malformation or suboptimal evaluation during routine anomaly scan. To identify all reasons for fetal echocardiography requests, detailed obstetric USG reports of each case were retrieved from the system and examined, and the reasons for fetal echocardiography requests were determined as accurately as possible by comparing these with our records. Pre-gestational and gestational medical data of pregnant women were retrieved. Comorbid medical problems that may be considered as risky for CHD, medication use, and characteristics of previous pregnancies were recorded. The pregnancies were categorized into two groups based on the risk of CHD: low-risk and high-risk groups. Fetal cardiac evaluation was performed by the same single operator (P.K) in the Department of Pediatric Cardiology using a C5-1 convex probe with an Affinity 70 (Philips, WA, USA) device. In all fetal echocardiography examinations, cardiac situs, size and position, systemic and pulmonary venous connections, atrial and ventricular chambers, atrioventricular and semilunar valves, ventriculoatrial shunts, great vessels outflow tracts, ductal and aortic arches, and rhythm were evaluated. If all views could not be clearly evaluated or if a suspicious finding is detected in the first evaluation, repeated evaluations may be required. Echocardiography images and reports of each patient were recorded. Fetal echocardiographic evaluation was scheduled according to the gestational week of the pregnancy, the referral diagnosis of the fetus, and the availability of our out-patient clinic. Each case was informed about applying for postnatal cardiac evaluation even if no pathology was detected in the fetal evaluation. For the purpose of performing as many evaluations and as early as possible, postnatal echocardiography evaluations are performed without appointment in our clinic. The postnatal data of 261 infants were retrieved of 714 single and 22 sets of twins, and 758 infants in total from 736 pregnancies were examined. Prenatal and postnatal echocardiography data of 261 cases, for which postnatal data could be retrieved, were compared.

Detected congenital cardiac structural malformations were classified as complex, moderate, and mild according to perinatal mortality risk (Table 1)[6,7]. Malformations that are not included in this classification, and malformations with low mortality risk

Table 1. Classification of prenatally diagnosed congenital heart defects

Complex; atresia or severe hypoplasia of a valve or chamber	Heterotaxy or atrial isomerism, single ventricle, hypoplastic left heart, pulmonary atresia, tricuspid atresia, aortic atresia, mitral atresia, and Ebstein's anomaly, complet atrioventricular septal defect, truncus arteriosus, congenitally corrected transposition of the great arteries, double outlet left or right ventricle
Modere; congenital heart disease requiring operation or intervention, but not included in the complex group	Transposition of the great vessels, tetralogy of Fallot, coarctation of the aorta, aortopulmonary window, critical aortic or pulmonary stenosis, partial atrioventricular septal defect, total anomalous pulmonary venous connection, large ventricular septal defect
Minor; no intervention	Small ventricular septal defect, atrial septal defect and less severe aortic or pulmonary stenosis
Others	Dysrhthmias, cardiomyopathies, secondary dextrocardia/levocardia and restrictive ductus

were evaluated in the others category. This study was approved by the Eskişehir Osmangazi University Institutional Ethics Committee.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences, version 15 (SPSS, Chicago, USA). The pregnancies were categorized into two groups based on the risk of CHD. The fetal echocardiography request reasons and the congenital cardiac malformation rates were classified according to pregnancy risk profile. The prevalence of CHDs in low- and high- risk pregnancies was compared using the chi-square test. Statistical significance was inferred at $p<0.05$.

Results

The mean age of the mothers was 29.8±5.6 years (range, 17-47 years), and the mean gestational age at which the fetal echocardiography examination was performed was 26.4±4.4 weeks. Of the 736 pregnancies, 22 were twin pregnancies, and fetal cardiac evaluation was performed in 758 fetuses. Twelve patients underwent examination for a second time. In total, 37.9% of the examinations were performed before the 24th week of pregnancy, while 62.1% were performed after the 24th week of pregnancy. Number of fetuses with congenital structural cardiac malformation was 101 (13.7%), and the number of fetuses with arrhythmia was 15 (2%).

The reasons for fetal echocardiography requests were classified according to CHD risk profiles considering the American Heart Association recommendations (Table 2)[8]. The prevalence of CHD according to risk factors was determined. There were 341 (46.3%) pregnancies in the high-risk group and 395 (53.6%) pregnancies in the low-risk group. The most common cause for fetal cardiac evaluation request was inability to adequately visualize the fetal heart (36.1%), while suspected fetal cardiac abnormality was the second most common cause (21.3%). Fetal cardiac malformation was detected as the most frequent among pregnant women referred to the pediatric cardiology due to suboptimal evaluation during routine anomaly scan (37.5%).

The distribution of CHDs, which were diagnosed with fetal echocardiography and classified according to mortality risk according to pregnancy risk groups was presented in Table 3. Number of fetuses detected with cardiac abnormalities was 80 (23.5%) among high-risk pregnancies, and 20 (5%) among low-risk pregnancies. The prevalence of cardiac abnormalities in each category was higher in high-risk pregnancies (Table 4). The most common type of malformation was simple cardiac abnormalities (6%) followed by complex lesions (4.1%). The most common cardiac abnormality was ventricular septal defect (VSD) comprised of 18 cases (2.4%) while the most common complex cardiac abnormality was pulmonary atresia (PA) (1.2%). The moderate cardiac abnormality was TOF including nine cases (1.2%). Out of 20 fetuses (2.7%) evaluated with suspected fetal cardiac arrhythmia, two had complete atrioventricular block, two fetuses had blocked premature atrial contractions, and two fetuses had supraventricular tachycardia. The other nine fetuses had premature atrial and ventricular contractions, which recovered during the late weeks of pregnancies.

The number of fetuses for which postnatal cardiac evaluation records could be retrieved via echocardiography examinations performed after birth, autopsy reports of terminated fetuses, and late period presentations to our clinic for follow-up purposes was 261 (35.4%). Of the 261 infants, 43 (16.4%) had CHD. As the prenatal and postnatal results were compared, there were 24 (9.1%) discordant diagnoses: One major and 23 minor. In one fetus for which fetal echocardiography was requested because of the presence of a sibling with heart disease and which did not have fetal cardiac abnormalities, total anomalous pulmonary venous return was detected in the first postnatal week. Twenty-three minor discordant diagnoses [15 atrial septal defect (ASD)], 4 VSD, 1 bicuspid aortic valve, 3 pulmonary stenosis] belonged to the class of simple heart diseases.

Discussion

Diagnosis of CHD during the intrauterine period provides significant benefits as performing of the birth under appropriate conditions, mentally prepared family, defining potential genetic abnormalities, and termination of pregnancy in the presence of complex malformations. Moreover, it was reported that diagnosis of some specific CHDs during the prenatal period increases the survival rate as well[9]. However, due to long duration of fetal echocardiography and requirement of an experienced pediatric cardiologists, it is not routinely performed

Table 2. The distribution of the fetal echocardiography request reasons and the congenital cardiac malformation rates according to pregnancy risk profile*

Indications with higher risk profile	Total n (%)	CHD n (%)
Gestational diabetes	30 (4.1)	2 (6.7)
Pregestational diabetes	27 (3.7)	2 (7.4)
Collagen tissue disease (maternal autoantibodies)	6 (0.8)	1 (20)
CHD in the first degree relatives of the fetus (mother, father, siblings)	25 (3.4)	0 (0)
Fetal cardiac abnormality suspected on obstetrical ultrasound	157 (21.3)	63 (40.1)
Fetal extracardiac abnormality suspected on obstetrical ultrasound	20 (2.7)	3 (15)
Fetal tachycardia or bradycardia, or frequent or persistent irregular heart rhythm	20 (2.7)	1 (5)
Fetal increased NT >95% (≥3 mm)	16 (2.2)	1 (6.25)
Monochorionic twinning	22 (3)	3 (13.6)
Fetal hydrops or effusions	13 (1.7)	2 (15.3)
Polyhidramnios	4 (0.5)	2 (50)
Total	341 (46.3)	80 (23.5)
Indications with lower risk profile		
Maternal medications (anticonvulsants, NSAIDS in first/second trimester)	28 (3.8)	1 (3.6)
Fetal abnormality of the umblical cord or placenta	19 (2.6)	2 (10.5)
Abnormal first or second trimester screening tests	41 (5.6)	2 (4.9)
Oligohydramnios	6 (0.8)	1 (16.6)
Late maternal age	35 (4.8)	3 (8.6)
Suboptimal evaluation	266 (36.1)	11 (4.1)
Total	395 (53.6)	20 (5)

CHD: Congenital heart disease, NSAIDS: Non-steroidal anti-inflammatory drug, NT: Nuchal translucency

in all pregnant women. Thus, basic fetal cardiac evaluation has become a part of the USG in routine obstetric monitoring. CHDs can be detected during the intrauterine period at a rate of 4.5%-8.1% with the evaluation of the fetal heart in four chamber view and at 43.8%-85.5% with the additional examination of the right and left ventricular outflow tracts[9]. Therefore, the prevalence of intrauterine diagnosis of CHD varies according to the protocol performed by the centers for fetal cardiac evaluation[10,11]. In the present study consisting of an eighteen-month period, fetal congenital cardiac malformation prevalence was 13.7%. Fetal congenital cardiac malformation prevalence was reported to be 5.6% from another tertiary center in Turkey[12]. At our center, evaluation of the fetal heart in four chamber view is a routine part of the obstetric USG. Moreover, detailed fetal cardiac evaluation including right and left ventricular outflow tracts and three vessels and trachea view is routinely performed in each pregnant woman by skilled perinatologists between the 18th and 22nd weeks of pregnancy. Furthermore, since the fetal echocardiography was performed in selected pregnancies who were identified as risky in antenatal screening in our tertiary reference center, the reported prevalence may be higher than expected.

In the present study, while suspected fetal cardiac abnormality was the second most common (21.3%) reason for fetal echocardiography request, inadequate evaluation of the fetal heart was the most common reason (36.1%). Prevalence of family history of CHD and maternal diabetes mellitus (DM), which were reported to be the top two most common reasons for fetal echocardiography requests in the previous studies, were 3.4% and 7.8%, respectively in the present study[10,13]. In the present study, similar to Cha et al.[14], detection of an abnormal cardiac finding during obstetric follow-up was more common than family history of CHD and maternal DM among the reasons for fetal echocardiography requests. Compatibility between the findings of the pediatric cardiologist and the obstetrician in the cases referred to fetal echocardiography with suspected CHD by the obstetricians varies according to the experience of the centers. In the current study, the rate of consistency was 40.1% between obstetricians and pediatric cardiologist in terms of the diagnosis of the congenital cardiac malformations. Simpson[15] reported that cardiac malformation was detected during fetal echocardiography in 45 of 275 (16%) pregnant women referred with suspected CHD, while Meyer-Wittkopf et al.[16] demonstrated that cardiac abnormality was detected in 209 out of 268 (77.9%) pregnant women referred to fetal echocardiography with suspected fetal cardiac abnormality, and the diagnosis was fully compatible in 62% of

Table 3. Intrauterine detected congenital heart diseases and their distribution according to pregnancy risk groups*

Results of fetal echocardiography	High risk group (n)	Low risk group (n)	Total n (%)
Complex			
PA with IVS/VSD/AVSD	9	0	9 (1.2)
Single ventricule	2	0	2 (0.3)
HLHS	6	0	6 (0.8)
DORV	3	1	4 (0.5)
Ebstein's anomaly	2	0	2 (0.3)
Idiopatic diffuse calsification	1	0	1 (0.1)
Shone complex	2	0	2 (0.3)
Left atrial isomerism	2	0	2 (0.3)
AVSD	2	0	2 (0.3)
Total	29	1	30 (4.1)
Modere			
TOF	7	2	9 (1.2)
CoA	5	1	6 (0.7)
d-TGA	4	0	4 (0.5)
Total	16	3	19 (2.4)
Simple			
VSD	14	4	18 (2.4)
Possible CoA	4	3	7 (1)
PS	0	3	3 (0.4)
AS	8	2	10 (1.4)
ASD	2	3	5 (0.7)
Total	28	15	43 (6)
Other			
Tricuspid regurgitation	1	0	1 (0.1)
LPVCS	3	0	3 (0.4)
Intracardiac Mass	2	0	2 (0.3)
Double aortic arch	1	0	1 (0.1)
Dextrocardia	1	0	1 (0.1)
Dysrhythmias	14	1	15 (2)
Total	22	1	23 (3)

CoA: Coarctation of aorta, AS: Aort stenosis, ASD: Atrial septal defect, AVSD: Atrio ventricular septal defect, DORV: Double outler right ventricle, HLHS: Hypoplastic left heart syndrome, IVS: Intact ventricular septum, LPVCS: Left persistant vena cava superior, PA: Pulmonary atresia, PS: Pulmonary stenosis, TGA: Transposition of great vessels, TOF: Tetralogy of Fallot; VSD: Ventricular septal defect
*The prevalence of cardiac abnormalities in each category was higher in high-risk pregnancies. According to the chi-square test, p<0.001

these patients. Obstetricians' increasing experience in evaluating fetal heart enables us to diagnose a higher rate of CHD during the intrauterine period.

When pregnant women that underwent fetal echocardiography were classified according to risk levels in terms of fetal cardiac malformation; 46.3% were in the high-risk group, and 53.6% were in the low-risk group. In fetal echocardiography, CHD was detected most commonly in the high-risk group with a rate of 23.5%. In the low-risk group, determination of congenital heart abnormality rate was 5%, and it was significantly higher among high-risk pregnancies. This was associated with the detection of cardiac malformation in 40.1% of pregnancies referred to fetal echocardiography due to suspected fetal cardiac malformation in the obstetric USG. In the study of Nayak et al.[9], in which fetal echocardiography was performed in all pregnant women over a period of 4 years, they highlighted the importance of fetal echocardiography. They concluded that the fetal echocardiography should be included as a part of routine antenatal screening irrespective of risk factors for CHD. According to the their results, the prevalence of prenatal CHD was similar between high- and low-risk pregnancies, but the majority of pregnancies with cardiac malformation was in the low-risk group[9], which is contrary to our finding. Operator misevaluation, failure to notice cardiac malformation, and inadequacy of and failure to interpret fetal cardiac image views were given as the reasons for failure to detect complex-structured fetal cardiac malformations in obstetric USG. The missing diagnoses in these pregnancies, which were referred with low-risk, were made with fetal echocardiography[9]. Evaluation of fetal heart in obstetric USG requires experience and knowledge, and obstetric fetal cardiac evaluation which is not optimally performed owing to various reasons can cause complex cardiac malformations to be missed. Therefore, we find it appropriate to refer these pregnant women in whom fetal heart could not be adequately evaluated by obstetric USG with reasons similar to those stated by Nayak et al.[9] to fetal echocardiography in our center as well. According to our results, inadequate evaluation of fetal heart in obstetric USG is the most common (31%) reason for requesting fetal echocardiography. However, fetal cardiac malformation was detected only in 4.1%. This was associated to a large extent with high-risk pregnancies in which obstetric fetal cardiac evaluation was conducted by an experienced perinatologist.

Table 4. Comparison of pregnancy risk groups in terms of fetal echocardiography results

Fetal cardiac anomaly	High risk group n (%)	Low risk group n (%)	p*
Complex	29 (8.5)	1 (0.25)	
Modere	16 (4.7)	3 (0.9)	
Simple	28 (8.4)	16 (4.2)	<0.001
Other	22 (6.2)	1 (0.24)	

*chi-square test

It is known that the prevalence of CHD differs during the intrauterine and postnatal periods. Isolated VSD is the most common CHD during the postnatal period, which is also reported to be the most common CHD diagnosed during the

intrauterine period as well [12,17]. In the present study, consistent with the literature, the most common cardiac malformation in fetal echocardiography is VSD. In the previous studies, complex cardiac malformations were reported to be the most common group of CHD[9,12,18]. However, we found complex cardiac malformations as the second most common following minor lesions. It was considered that this variation may be related to some differences in the classification of cardiac lesions between the studies. Moreover, in the present study, consideration of the higher but insignificant increases in flow velocities at semilunar valves may have played a role in the increased number of minor lesions. In previous studies, atrioventricular septal defect (AVSD) and hypoplastic left heart syndrome (HLHS) were reported to be complex cardiac lesions that are detected at a similar or higher prevalence[12,18,19]. In the present study, the most common complex CHD was PA, followed by HLHS.

Since the pregnancies diagnosed with complex type congenital cardiac malformations were referred to the surgical center during the prenatal or postnatal period, and the others diagnosed as moderate type lesions gave birth in our hospital, postnatal results of all of these pregnancies were retrieved. Moreover, most of the 261 (35.4%) cases, whose postnatal echocardiography results could be retrieved, were diagnosed with fetal cardiac abnormality by an obstetrician or pediatric cardiologist. The incompatibility between prenatal and postnatal echocardiography in these 261 cases were mostly due to simple lesions. It was found that the diagnoses did not change in complex lesions which were detected by a pediatric cardiologist, but one case with a normal fetal echocardiography was diagnosed with total anomalous pulmonary venous return abnormality during the postnatal period. Pulmonary venous return abnormalities, small or moderate sized ventricular or ASD, and minor valve lesions cannot always be defined with fetal echocardiography, and are frequently diagnosed after birth[8]. Meyer-Wittkopf et al.[16] also reported that a total anomalous pulmonary venous return abnormality diagnosed in the postnatal period could not be detected in the fetal echocardiography.

Study Limitations

Although each evaluated pregnant woman was informed about the importance of postnatal echocardiography, a significant amount of postnatal echocardiographic evaluation, in which a statistical analysis for sensitivity and specificity could not be performed in order to make a confirmation or comparison.

Conclusion

In many centers, regardless of CHD risk, it is still not possible to perform fetal echocardiography by a pediatric cardiologist to all pregnant women. Therefore, routine evaluation of the fetal heart by means of obstetric USG, including four chambers, outflow tracts' and three vessel views, would allow for diagnosing congenital cardiac malformations to a large extent during the intrauterine period.

Ethics

Ethics Committee Approval: This study was approved by the Eskişehir Osmangazi University Institutional Ethics Committee (approval no: 25403353-050.99).
Informed Consent: The study is retrospective.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: P.K., T.K., H.K., M.V., B.U., Concept: P.K., M.V., B.U. Design: P.K., M.V., B.U., Data Collection or Processing: P.K, T.K., H.K., M.V., Analysis or Interpretation: P.K, T.K., H.K., M.V., B.U., Literature Search: P.K., M.V., Writing: P.K., M.V.
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Pulmonary morbidity related to diaphragm surgery performed for gynecological cancers

Jinekolojik kanser hastalarında yapılan sitoredüktif cerrahiler kapsamında gerçekleştirilen diyafram cerrahilerinin pulmoner morbidite ile ilişkisi

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Abstract

Objective: To evaluate pulmonary morbidity related to diaphragm surgery performed for gynecological cancers and to identify the impact of transdiaphragmatic thoracotomy.

Materials and Methods: We reviewed clinical and pathologic data of 232 women who had undergone diaphragm surgery as a part of cytoreductive surgery procedures performed for gynecological cancers.

Results: Transdiaphragmatic thoracotomy occurred in 52 patients (22.4%). Rate of pulmonary complications among patients who had a transdiaphragmatic thoracotomy was higher compared with patients who did not have a transdiaphragmatic thoracotomy (40.4% vs 20.6%, p=0.004). Transdiaphragmatic thoracotomy [odds ratio (OR), 2.66; 95% confidence interval (CI), 1.20-5.92; p=0.016], colon resection (OR, 5.21; 95% CI, 2.34-11.63; p<0.001), ileostomy (OR, 19.61; 95% CI, 1.64-250.0; p=0.019), and any extra-pulmonary complication occurrence (OR, 2.35; 95% CI, 1.13-4.88; p=0.023) were identified as independent predictors of pulmonary morbidity. Patients with transdiaphragmatic thoracotomy developed pleural effusion, pleural effusion necessitating drainage, pneumothorax, pneumonitis, and atelectasis more frequently compared with patients who did not have transdiaphragmatic thoracotomy. Rate of admission to postoperative intensive care of patients with transdiaphragmatic thoracotomy (30.8%) was significantly higher than that of patients without transdiaphragmatic thoracotomy (12.2%) (p=0.001).

Conclusion: Transdiaphragmatic thoracotomy is an independent predictor of pulmonary morbidity among patients who underwent diaphragm surgery. Avoiding accidental transdiaphragmatic thoracotomies with maximal attention and performing full-thickness resection procedures with alternative surgical techniques preventing a thoracotomy may help decrease pulmonary morbidity rates and postoperative care costs.

Keywords: Diaphragm, surgery, cytoreduction, ovarian cancer, morbidity

Öz

Amaç: Jinekolojik kanser hastalarında, sitoreduktif cerrahi kapsamında uygulanan diyafram cerrahisi ile ilişkili pulmoner morbiditenin değerlendirilmesi.

Gereç ve Yöntemler: Sitoredüktif cerrahi kapsamında diyafram cerrahisi de uygulanan 232 jinekolojik kanser hastasına ait veriler retrospektif olarak tarandı.

Bulgular: Transdiyafram torakotomi 52 (%22,4) hastada oluşmuştu. Transdiyafram torakotomi olan hastalarda herhangi bir pulmoner komplikasyon ortaya çıkma oranı %40,4 iken, transdiyafram torakotomi olmayan hastalarda herhangi bir pulmoner komplikasyon ortaya çıkma oranı %20,6 idi. Transdiyafram torakotomi olan hasta grubunda pulmoner komplikasyon gelişme riskinin daha yüksek olduğu görüldü (p=0,004). Transdiyafram torakotomi [risk oranı (OR), 2,66; %95 güven aralığı (CI), 1,20-5,92; p=0,016], kolon rezeksiyonu (OR, 5,21; %95 CI, 2,34-11,63; p<0,001), ileostomi (OR, 19,61; %95 CI, 1,64-250,0; p=0,019), ve herhangi bir extra-pulmoner komplikasyon oluşmuş olması (OR, 2,35; %95 CI, 1,13-4,88; p=0,023) pulmoner morbiditenin bağımsız prediktörleri olarak tanımlandı. Pulmoner morbidite alt başlıklar halinde değerlendirildiğinde; transdiyafram torakotomi olan hasta grubunda plevral efüzyon, drenaj gerektiren plevral efüzyon, pnomotoraks, pnomoni ve atelektazinin, transdiyafram torakotomi olmayan hastalar ile karşılaştırıldığında, daha sık ortaya çıktığı gösterildi. Yoğun bakım ünitesi ihtiyacı oranı, transdiyafram torakotomili hasta grubunda, transdiyafram torakotomisi olmayan hasta grubuna kıyasla daha yüksekti (%30,8 vs. %12,2, p=0,001).

PRECIS: Transdiaphragmatic thoracotomy, colon resection, ileostomy and development of any extra-pulmonary complication were identified as independent predictors of pulmonary morbidity.

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Sonuç: Transdiyafram torakotomi, diyafram cerrahisi geçiren hastalarda pulmoner morbiditenin bağımsız bir prediktörüdür. Maksimal özen ile accidental trasdiyafram torakotomilerden kaçınmak ve tam kat diyafram rezeksiyonlarını torakotomi oluşturmayan alternatif cerrahi yöntemler ile gerçekleştirmek pulmoner morbidite oranının azaltılmasına ve postoperatif bakım masraflarının azaltılmasına yardımcı olabilir.
Anahtar Kelimeler: Diyafragm, cerrahi, sitoredüktif, over kanseri, morbidite

Introduction

Studies have shown that optimal cytoreduction is the cornerstone of initial treatment for advanced epithelial ovarian cancers[1,2]. It is estimated that nearly 40% of patients with advanced ovarian cancer have gross disease located on the diaphragm[3,4]. Patients with advanced ovarian cancer and diaphragm involvement who had undergone diaphragm surgery had better survival rates than those who had not[5]. Thus, a significant proportion of patients with advanced ovarian cancers require diaphragm surgery to achieve optimal cytoreduction and therefore better survival rates. Complete cytoreduction is also associated with improved overall survival in advanced uterine corpus cancers, and patients with metastatic diaphragm disease require diaphragm surgery[6,7].

Previous studies reported that diaphragm surgery was associated with pulmonary morbidity, and they reported higher rates of pleural effusion[4,8-12], higher rates of pneumothorax[8,10-12] and longer hospital stay[4] after diaphragm surgery. Furthermore, several studies showed higher rates of pulmonary morbidity after diaphragm full-thickness resections compared with diaphragm peritoneal stripping[13,14] ,but others did not show a significant difference[9,15]. Previous studies were not able to assess other types of pulmonary morbidity such as pneumonitis and atelectasis due to the small number of cases. Thus, pulmonary morbidity related to diaphragm surgery needs further evaluation with a larger number of cases. We performed the present study to improve knowledge about pulmonary morbidity related to diaphragm surgery, and we also assessed other potential surgical factors that may contribute to pulmonary morbidity.

Materials and Methods

All patients who had undergone diaphragm surgery as a part of cytoreductive surgery procedures performed for advanced or recurrent ovarian and uterine corpus cancers between January 1, 2001, and June 1, 2018, were reviewed retrospectively. Data were obtained from hospital medical records. Patients who had an ablative intervention to the diaphragm were excluded from the study. We included only cases involving surgical resections of the diaphragm peritoneum with or without diaphragm muscle and overlying parietal pleura. Patients with preoperative pleural effusions and intraoperative chest tube replacements would lead to unreliable evaluation of the postoperative pulmonary complications so they were excluded.

Diaphragm debulking surgery to excise all visible metastatic tumoral deposits on the diaphragm surface was performed primarily by excision of the diaphragm peritoneum (peritoneal stripping). The extent of the diaphragm peritoneal excision was classified as "total hemi-diaphragm peritoneal stripping", "partial hemi-diaphragm peritoneal stripping", or "focal implant resection." Excision of the whole hemi-diaphragm peritoneum starting from the anterior costal margin was identified as "total hemi-diaphragm peritoneal stripping". Excision of ≤3 tumor implants ≤2 cm in diameter was identified as "focal implant resection". All the other peritoneal excisions that were not compatible with the above definitions were classified as "partial hemi-diaphragm peritoneal stripping".

Full mobilization of the liver was performed routinely before starting a right hemi-diaphragm total peritoneal stripping procedure. Liver mobilization was performed when needed for partial hemi-diaphragm stripping or a focal implant resection procedure depending on the location of the tumor. Tumor involvement on the left hemi-diaphragm is more easily resected without mobilizing the liver.

Diaphragm surgery may be complicated by transdiaphragmatic thoracotomy. In our study, we classified the cause of transdiaphragmatic thoracotomy as either "willful partial hemi-diaphragm full-thickness resection" or "accidental transdiaphragmatic thoracotomy". If the surgical report indicated a histologically confirmed tumor implant invading the diaphragm muscle with or without parietal pleura, we identified the cause of the transdiaphragmatic thoracotomy as "willful partial hemi-diaphragm full-thickness resection". If not, we identified the cause as "accidental transdiaphragmatic thoracotomy".

Diaphragm repair was done by continuous suturing of the defect with 2/0 prolene sutures. Sutures were tied while the anesthetist was performing a forced inspiration with the help of the ventilator and the surgical team was applying a negative pressure to the thorax cavity with the help of a thin aspiration tube positioned through the remaining diaphragm defect. The second surgeon pulled out the aspiration tube as the first surgeon tied the knot. Large diaphragm defects required repair with prolene mesh.

All patients received preoperative antibiotics and anticoagulant prophylaxis. No prophylactic antibiotics were used postoperatively. Enoxaparine 4000 international units (IU) was administered 2 hours before the surgery and then repeated daily for 30 days after the surgery if the body mass index of the patient was <40 kg/m^2; enoxaparine 6000 IU was administered if the body mass index was ≥40 kg/m^2. All patients were assessed with a chest radiography on the first postoperative day and with physical examination daily during hospitalization. Chest radiography was repeated on the following days if any pathologic signs were detected on the physical examination. Chest computed tomography was performed when needed. Admission to the postoperative general care room or the

intensive care unit was carried out according to the decision of the anesthetist. Replacement of the chest tube in the postoperative period was decided and performed by the thorax surgeon when needed. Referral to the intensive care unit during postoperative general care was decided by the senior surgeon and anesthetist when needed.

We reviewed intraoperative complications and postoperative complications that occurred within 30 days. Pulmonary morbidity was identified as the development of at least one of the following pulmonary complications: Pleural effusion, pneumothorax, pneumonitis, atelectasis, pulmonary embolism. Extra-pulmonary complications in the study group included wound infection, wound dehiscence, evisceration, intestinal anastomosis leak, ureterovaginal fistulas, vesicovaginal fistulas, gastrointestinal fistulas, ileus, acute cerebrovascular disease, chylous ascites, intraabdominal abscess, subphrenic abscess, intestinal perforation, gastric perforation, bladder perforation, pancreatic leak/fistula, appendectomy site leak, intestinal ischemia, ureter ischemia, deep venous thrombosis, arterial embolism, and myocardial infarction.

This study was approved by institutional review board (approval date and number: 21.12.2018/ 90057706-799-E.8). Signed informed consent for anonymous publication of disease related information was obtained from each patient.

Statistical Analysis

Data were analyzed using the chi-squared test and Fisher's Exact test for categorical variables, and the independent sample t-test was used for continuous variables. A binary logistic regression model was used for the multivariate analysis. Differences were considered significant at $p<0.05$. Statistical Package for Social Science (SPSS, version 17.0, SPSS, Inc., Chicago, IL, USA) was used for the statistical analyses.

Results

We included 232 patients who had undergone diaphragm surgery. The mean age was 60.14±9.47 years (range, 36-82 years). Two hundred nine patients (90.1%) had ovarian-tubal-peritoneal cancers, 18 (7.7%) had uterine corpus cancers, 5 (2.1%) had synchronized cancers. The histopathologic subgroups are summarized in Table 1. One hundred ninety-two patients (82.8%) had undergone diaphragm surgery within the context of primary cytoreduction surgery. Forty patients (17.2%) had undergone diaphragm surgery because of recurrent cancers. Cytoreductive surgery resulted in excision of all visible tumors in 213 patients (91.8%).

One hundred eighty-three patients (78.9%) had undergone right hemi-diaphragm surgery, 2 patients (0.9%) had undergone left hemi-diaphragm surgery, and 47 patients (20.2%) had undergone bilateral hemi-diaphragm surgery. One hundred sixty-two patients (69.8%) had undergone a total hemi-diaphragm peritoneal stripping procedure, 9 patients (3.9%) had undergone a partial hemi-diaphragm peritoneal stripping procedure, 61 patients (26.3%) had undergone focal implant resection. The surgical notes reported presence of ascites in 96 patients (41.4%). Mean intraoperative blood loss was 808.3±928.1 mL. Mean operation time was 336.3±69.6 minutes. Thirty-eight patients (16.4%) needed postoperative intensive care. The median hospital stay was 9 days, and the

Table 1. Histopathologic characteristics of the study group

Histopathology	Number (%)
Ovarian-tubal-peritoneal cancers	209 (90.1)
Ovarian serous adenocarcinoma	157 (67.7)
Ovarian endometrioid adenocarcinoma	13 (5.6)
Ovarian clear cell adenocarcinoma	3 (1.3)
Ovarian transitional cell adenocarcinoma	4 (1.7)
Ovarian borderline mucinous tumor	1 (0.4)
Ovarian mixed epithelial cancers	6 (2.6)
Tubal serous adenocarcinoma	3 (1.3)
Peritoneal serous adenocarcinoma	10 (4.3)
Immature teratoma	2 (0.9)
Granulosa cell tumor	2 (0.9)
Tubal carcinosarcoma	1 (0.4)
Ovarian carcinosarcoma	7 (3.0)
Uterine corpus cancers	18 (7.7)
Endometrium endometrioid adenocarcinoma	5 (2.2)
Endometrium serous adenocarcinoma	6 (2.6)
Endometrium undifferentiated adenocarcinoma with neuroendocrine differentiation	1 (0.4)
Uterine carcinosarcoma	3 (1.3)
Low-grade endometrial stromal sarcoma	1 (0.4)
Uterine tumor resembling ovarian sex-cord tumor	1 (0.4)
Uterine leiomyosarcoma	1 (0.4)
Synchronized cancers	5 (2.1)
Synchronized right ovarian serous adenocarcinoma-left ovarian granulosa cell tumor	1 (0.4)
Synchronized ovarian serous adenocarcinoma + endometrium endometrioid adenocarcinoma	1 (0.4)
Synchronized ovarian endometrioid adenocarcinoma + endometrium endometrioid adenocarcinoma	2 (0.9)
Synchronized ovarian mucinous adenocarcinoma + cervical large cell neuroendocrine carcinoma	1 (0.4)

median time from surgery to the first chemotherapy cycle was 15 days. No complications occurred in 133 patients (57.3%). Twenty-nine patients (12.5%) had pulmonary complications only, 41 patients (17.7%) had extra-pulmonary complications only, and 29 patients (12.5%) had both pulmonary and extra-pulmonary complications. Three patients died within the 30-day period after surgery, and the mortality rate was 1.3%. One patient died because of aspiration pneumonitis and 2 died as a result septic complications.

In our study, accidental transdiaphragmatic thoracotomy occurred in 20 patients (8.6%). Willful partial full-thickness excision of the hemi-diaphragm was performed in 33 patients (14.2%). One willful partial hemi-diaphragm full-thickness resection procedure was performed with a gastrointestinal staple device avoiding an entry into the pleural cavity so she was not included in the transdiaphragmatic thoracotomy group, thus remaining 52 patients (22.4%) formed the transdiaphragmatic thoracotomy group. In the study period, one large diaphragm defect required repair with prolene mesh.

We analyzed all clinicopathologic and surgical factors with regard to pulmonary morbidity. There was a higher rate of pulmonary complications when the surgical notes reported the presence of transdiaphragmatic thoracotomy. Twenty-one patients (40.4%) who had a transdiaphragmatic thoracotomy developed a pulmonary complication. Thirty-seven patients (20.6%) who did not have a transdiaphragmatic thoracotomy developed a pulmonary complication postoperatively. The difference was statistically significant (p=0.004) (Table 2). According to our data, patients who developed pulmonary complications and patients who did not develop pulmonary complications showed insignificant difference with regard to age. The mean age of patients who developed pulmonary complications was 57.4±7.9. The mean age of patients who did not develop pulmonary complications was 60.9±9.7. The difference between two groups was insignificant (p=0.108).

Patients who underwent small-bowel resection, colon resection, pelvic peritonectomy, appendectomy, tumor resection from liver Glisson's capsule, tumor resection from the omentum minus, tumor resection from Morrison's pouch, tumor resection from colon serosa, ileostomy, colostomy concurrently had significantly higher rates of pulmonary complication. The mean intraoperative blood loss of the patients who developed pulmonary complications was significantly higher than that of patients who did not develop any pulmonary complications (p=0.045). Patients who developed an extra-pulmonary complication had significantly higher rate of pulmonary complications compared with those who did not (p<0.001). Patients who developed an intestinal anastomosis leak had significantly higher rate of pulmonary complications (p=0.006) (Table 2).

The factors associated with pulmonary morbidity are summarized in Table 2, and we analyzed them with a binary logistic regression model. We found that transdiaphragmatic thoracotomy [odds ratio (OR), 2.66; 95% confidence interval (CI), 1.20-5.92; p=0.016], colon resection (OR, 5.21; 95% CI, 2.34-11.63; p<0.001), ileostomy (OR, 19.61; 95% CI, 1.64-250.0; p=0.019), and any extra-pulmonary complication occurrence (OR, 2.35; 95% CI, 1.13-4.88; p=0.023) were independent predictors of pulmonary morbidity (Table 3).

In our study, 48 patients (20.7%) developed pleural effusions postoperatively; 19 patients (8.2%) developed pleural effusions that necessitated drainage; 6 patients (2.6%) developed pneumothorax; 5 patients (2.1%) developed pneumonitis; 19 patients (8.2%) developed atelectasis; 5 patients (2.1%) developed pulmonary embolism. We divided the study population into 2 groups according to the absence or presence of transdiaphragmatic thoracotomy and compared the pulmonary complication subtypes separately. Patients in the transdiaphragmatic thoracotomy group developed pleural effusion, pleural effusion necessitating drainage, pneumothorax, pneumonitis, and atelectasis more frequently than patients in the no transdiaphragmatic thoracotomy group (Table 4).

Mean hospital stay, mean time interval from surgery to chemotherapy, and rate of postoperative admission to intensive care for patients with or without pulmonary complications and transdiaphragmatic thoracotomy are shown in Table 5. Patients who developed pulmonary complications had significantly longer hospital stay, longer time interval to chemotherapy, and needed postoperative intensive care more frequently. The differences in mean hospital stay and mean time interval to chemotherapy between the groups with and without transdiaphragmatic thoracotomy were insignificant. But the rate of admission to postoperative intensive care of patients with transdiaphragmatic thoracotomy was significantly higher than that of patients without transdiaphragmatic thoracotomy (Table 5).

Discussion

Diaphragmatic surgery is performed frequently within the context of cytoreductive surgery for advanced ovarian cancers, and cytoreduction to microscopic disease improves survival. Complete cytoreduction is also associated with improved overall survival in advanced uterine corpus cancers, and patients with metastatic diaphragm disease require diaphragm surgery. When diaphragm surgery is performed, it may cause pulmonary complications and additional morbidity[6,11]. In this study, 25% of patients developed a pulmonary complication. Pleural effusion was the most common complication and 20.7% developed pleural effusions postoperatively; 2.6% developed pneumothorax. Other studies reported pleural effusion rates after diaphragm surgery of between 2% and 59%, and pneumothorax rates between 0% and 5%[10,16,17].

Eisenhauer et al.[9] analyzed pleural effusions among 52 patients who had undergone diaphragm peritonectomy or resection. They reported that ipsilateral postoperative pleural effusions occurred in 30 patients (58%). Fifteen of the 52

Table 2. Factors associated with pulmonary morbidity

Factors		Development of pulmonary complications n (%)		p
		Yes	No	
Transdiaphragmatic thoracotomy	Yes	21 (40.4)	31 (59.6)	0.004
	No	37 (20.6)	143 (79.4)	
Unilateral versus bilateral diaphragm stripping	Unilateral	42 (22.7)	143 (77.3)	0.109
	Bilateral	16 (34.0)	31 (66.0)	
Total versus partial or focal diaphragm stripping	Total	46 (28.4)	116 (71.6)	0.069
	Partial/focal	12 (17.1)	58 (82.9)	
Transdiaphragmatic thoracotomy	Accidental	11 (55.0)	9 (45.0)	0.089
	Partial full-thickness resection	10 (31.3)	22 (68.8)	
Histopathology[a]	Ovarian-tubal-peritoneal invasive epithelial cancer	48 (24.5)	148 (75.5)	0.518
	Others	9 (30.0)	21 (70.0)	
Primary site[b]	Ovary-tuba-peritoneum	52 (24.9)	157 (75.1)	0.786
	Uterine corpus	5 (27.8)	13 (72.2)	
Cytoreduction	Primary	50 (26.0)	142 (74.0)	0.422
	≥2	8 (20.0)	32 (80.0)	
Preoperative Ca-125 (IU/mL), mean ± SD[c]		1328.70±1465.32	1151.96±1353.90	0.484
Intraoperative ascites	Yes	21 (21.9)	75 (78.1)	0.356
	No	37 (27.2)	99 (72.8)	
Ascites volume (mL), mean±SD		2447.62±2127.35	2760.26±2599.02	0.613
Suprahepatic drain	Yes	22 (28.2)	56 (71.8)	0.422
	No	36 (23.4)	118 (76.6)	
Duration of the suprahepatic drain (days), mean ± SD		5.9±4.7	4.6±2.4	0.111
Operation time (minutes), mean ± SD		340.7±76.9	335.6±68.8	0.796
Intraoperative blood loss (mL), mean ± SD		1132.6±1213.4	672.7±751.5	0.045
Small-bowel resection	Yes	13 (50)	13 (50)	0.002
	No	45 (21.8)	161 (78.2)	
Colon resection	Yes	46 (45.1)	56 (54.9)	<0.001
	No	12 (9.2)	118 (90.8)	
Pelvic peritonectomy	Yes	48 (31.6)	104 (68.4)	0.001
	No	10 (12.5)	70 (87.5)	
Appendectomy	Yes	47 (29.6)	112 (70.4)	0.018
	No	11 (15.1)	62 (84.9)	
Tumor resection from liver Glisson's capsule	Yes	31 (32.0)	66 (68.0)	0.038
	No	27 (20.0)	108 (80.0)	
Tumor resection from omentum minus	Yes	27 (32.9)	55 (67.1)	0.039
	No	31 (20.7)	119 (79.3)	

Table 2 continued				
Tumor resection from Morrison's pouch	Yes	22 (36.7)	38 (63.3)	0.015
	No	36 (20.9)	136 (79.1)	
Tumor resection from colon serosa	Yes	42 (29.8)	99 (70.2)	0.036
	No	16 (17.6)	75 (82.4)	
Ileostomy	Yes	7 (87.5)	1 (12.5)	<0.001
	No	51 (22.8)	173 (77.2)	
Colostomy	Yes	18 (56.3)	14 (43.8)	<0.001
	No	40 (20.0)	160 (80.0)	
Any extra-pulmonary complication	Yes	29 (41.4)	41 (58.6)	<0.001
	No	29 (17.9)	133 (82.1)	
Intestinal anastomosis leakage	Yes	7 (63.6)	4 (36.4)	0.006
	No	51 (23.1)	170 (76.9)	

All concurrent surgical procedures and extra-pulmonary complications were analyzed; only analyses with a p-value <0.05 are included in the table.
[a]One borderline mucinous tumor and 5 synchronized cancers were excluded from the analysis.
[b]Five synchronized cancers were excluded from the analysis.
[c]Only ovarian-tubal-peritoneal epithelial cancers subjected to primary cytoreduction surgery are included in the analysis

Table 3. Independent predictors of pulmonary morbidity

Independent predictors of pulmonary morbidity	Odds ratio	95% confidence interval	p
Transdiaphragmatic thoracotomy (performed versus not performed)	2.66	1.20-5.92	0.016
Colon resection (performed versus not performed)	5.21	2.34-11.63	<0.001
Ileostomy (performed versus not performed)	19.61	1.64-250.0	0.019
Any extra-pulmonary complication (positive versus negative)	2.35	1.13-4.88	0.023

Table 4. Comparison of pulmonary complications in patients with and without transdiaphragmatic thoracotomy

Pulmonary complication		No transdiaphragmatic thoracotomy group n (%)	Transdiaphragmatic thoracotomy group n (%)	p
Any kind of pulmonary complication	Yes	37 (20.6)	21 (40.4)	0.004
	No	143 (79.4)	31 (59.4)	
Pleural effusion	Yes	32 (17.8)	16 (30.8)	0.042
	No	148 (82.2)	36 (69.2)	
Pleural effusion, did not necessitate drainage	Yes	22 (12.2)	7 (13.5)	0.812
	No	158 (87.8)	45 (86.5)	
Pleural effusion, necessitated drainage	Yes	10 (5.6)	9 (17.3)	0.006
	No	170 (94.4)	43 (82.7)	
Pneumothorax	Yes	1 (0.6)	5 (9.6)	0.002
	No	179 (99.4)	47 (90.4)	
Pneumonitis	Yes	1 (0.6)	4 (7.7)	0.01
	No	179 (99.4)	48 (92.3)	
Atelectasis	Yes	11 (6.1)	8 (15.4)	0.032
	No	169 (93.9)	44 (84.6)	
Pulmonary embolism	Yes	4 (2.2)	1 (1.9)	>0.99
	No	176 (97.8)	51 (98.1)	

Table 5. Mean length of hospital stay, mean time from surgery to chemotherapy, and rate of postoperative intensive care

	Length of hospital stay (days), mean ± SD	p	Interval from surgery to chemotherapy (days), mean ± SD	p	Postoperative intensive care unit, n (%)		p
					Yes	No	
Pulmonary complication							
Yes	17.56±10.95	<0.001	20.48±11.14	0.023	27 (46.6)	31 (53.4)	<0.001
No	10.61±7.42		16.63±9.56		11 (6.3)	163 (93.7)	
Transdiaphragmatic thoracotomy							
Yes	13.33±8.85	0.376	18.83±10.96	0.344	16 (30.8)	36 (69.2)	0.001
No	12.05±8.95		17.17±9.81		22 (12.2)	158 (87.8)	

SD: Standard deviation

patients had undergone diaphragm resection or had diaphragm perforation. Eleven of 15 patients (73%) developed ipsilateral effusions. Although 73% of patients who underwent diaphragm resection and had perforations developed ipsilateral effusions, statistical analysis did not show a significantly increased rate of ipsilateral effusions after diaphragm resection or diaphragm perforation. The small number of patients in the study group and relatively high rates of pleural effusion may have led to the absence of statistical significance[9]. In our study, 20.7% of patients developed pleural effusions postoperatively. The rates of pleural effusion in patients without transdiaphragmatic thoracotomy and with transdiaphragmatic thoracotomy were 17.8% and 30.8%, respectively. Pleural effusion was more frequent among patients with transdiaphragmatic thoracotomy and it was statistically significant.

Soleymani Majd et al.[15] compared 64 cases with diaphragmatic peritonectomy and 36 cases with pleurectomy with regard to pulmonary morbidity. The rates of pulmonary morbidity in the peritonectomy group and the pleurectomy group were 9.3% and 19%, respectively, and there was no significant difference (p=0.14). They were able to show higher rates of pulmonary morbidity after pleurectomy compared with peritonectomy, but they were unable to show a statistically significant difference due to the limited number of cases[15]. Ye et al.[14] compared 124 patients with diaphragmatic peritonectomy and 26 cases with full-thickness diaphragmatic resection with regard to pulmonary morbidity, and they showed that patients who underwent full-thickness diaphragmatic resection developed pleural effusion significantly more frequently (25.8% versus 69.2%, p<0.001) and significantly more frequent symptomatic pleural effusion requiring drainage (8.9% versus 38.5%, p<0.001)[14]. Zapardiel et al.[13] compared 79 patients who underwent diaphragmatic stripping and 33 patients who underwent diaphragmatic resection and showed that patients with diaphragmatic resection developed pleural effusion significantly more frequently (37.9% versus 63.6%, p=0.01).

In the current study, we were able to identify transdiaphragmatic thoracotomy as an independent predictor of pulmonary morbidity after diaphragm surgery. To our knowledge, the present study is the largest case series evaluating pulmonary morbidity related to diaphragm surgery. We were able to show that patients with transdiaphragmatic thoracotomy developed pulmonary morbidity, pleural effusions, pleural effusions necessitating drainage, pneumothorax, pneumonitis, and atelectasis significantly more frequently than patients who underwent diaphragm surgery without transdiaphragmatic thoracotomy. In the current study, all the diaphragm surgery procedures and diaphragm repairment procedures were performed by gynecological oncologists and we think that diaphragm surgery procedures can be managed by experienced gynecological oncologists. A thorax surgeon should attend the operation when a pulmonary parenchymal resection is planned. Preoperative imaging with thorax computerized tomography may help to predict cases with thorax and diaphragm involvement.

Eisenhauer et al.[9] reported that ipsilateral pleural effusion was not associated with an increased length of hospital stay. Benedetti Panici et al.[4] analyzed 121 patients with advanced ovarian cancer. They performed diaphragm peritonectomy in 25 patients and diaphragm resections in 43 patients and reported that diaphragmatic resection was associated with significantly longer postoperative hospital stay (median, 10.6 versus 8.1 days, p=0.005). According to our data, development of any pulmonary morbidity was associated with longer hospital stay, longer time interval to chemotherapy, and a higher rate of postoperative intensive care. Patients with transdiaphragmatic thoracotomy needed postoperative intensive care more frequently than patients without transdiaphragmatic thoracotomy. Decreasing the rate of transdiaphragmatic thoracotomy among patients undergoing diaphragm surgery may help decrease postoperative intensive care admissions and therefore reduce postoperative care costs.

Development of an extra-pulmonary complication, concurrent colon resections, and ileostomy were also independent predictors of pulmonary morbidity. Among patients with concurrent colon resections and ileostomy, prolonged intravenous support and electrolyte imbalance may have contributed to higher rates of pleural effusions and pulmonary morbidity.

Study Limitations

The retrospective design of the study and the absence of a patient group with advanced gynecological cancer that did not undergo diaphragm surgery are limitations of the current study. The high number of patients in the study, the competence in evaluating pulmonary complications separately, and identifying independent factors associated with pulmonary morbidity by multivariate analyzes are advantages of the current study. To our knowledge, the present study is the largest case series evaluating pulmonary morbidity related to diaphragm surgery.

Conclusion

In conclusion, diaphragm surgery helps to enhance complete cytoreduction rates and therefore improves survival in advanced epithelial ovarian cancers and uterine corpus cancers. İt is associated with higher rates of pulmonary complications. Transdiaphragmatic thoracotomy is an independent predictor of pulmonary morbidity among patients who undergo diaphragm surgery. Avoiding accidental transdiaphragmatic thoracotomies with maximal attention may help decrease pulmonary morbidity rates and postoperative care costs. Potential benefits of alternative surgical techniques (e.g using staple devices) preventing entry into the thorax cavity while performing full-thickness diaphragm resections should further be investigated.

Ethics

Ethics Committee Approval: All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the Helsinki declaration and its later amendments or comparable ethical standards. Institutional Review Board approval was received for this study (approval date and number: 21.12.2018/ 90057706-799-E.8).

Informed Consent: Signed informed consent for anonymous publication of disease related information was obtained from each patient.

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Authorship Contributions

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Hemorrhagic corpus luteum: Clinical management update

Hemorajik korpus luteum: Klinik yönetimin güncellemesi

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Abstract

Hemorrhagic corpus luteum (HCL) is an ovarian cyst formed after ovulation and caused by spontaneous bleeding into a corpus luteum (CL) cyst. When HCL rupture happens, a hemoperitoneum results. Clinical symptoms are mainly due to peritoneal irritation by the blood effusion. The differential diagnosis is extensive and standard management is not defined. The authors elaborated a comparison of the differential diagnosis and therapeutic modalities from the laparoscopic approach to nonsurgical, medical options because hemorrhage from HCL is often self-limiting. The authors reviewed all data implicated with the development of HCL, trying to give homogeneity to literature data. The authors analyzed extensive literature data and subdivided the medical approach into many topics. The wait-and-see attitude avoids unnecessary laparoscopic surgery using supportive therapies (antifibrinolytic, analgesics, liquid infusion, transfusions and antibiotic prophylaxis). Surgical therapy: operative management should be laparoscopic, with surgical options such as luteumectomy, ovarian wedge-shaped excision or oophorectomy. Prevention: the possibility to preserve fertility is essential, mainly in patients with bleeding disorders or undergoing anticoagulant therapy; therefore, they need estro-progestinics or GnRH analogues to prevent ovulation and avoid further episodes of HCL. This review will aid physicians in making an early diagnosis of HCL, to avoid unnecessary surgery, and use the most effective treatment.
Keywords: Corpus luteum, ovarian cyst, ectopic pregnancy, laparoscopy

Öz

Hemorajik korpus luteum (HKL), yumurtlamadan sonra oluşan ve korpus luteum (KL) kisti içine spontan kanama nedeniyle oluşan bir yumurtalık kistidir. HKL rüptürü meydana geldiğinde hemoperitoneum oluşur. Klinik semptomlar esas olarak kan efüzyonunun neden olduğu periton tahrişine bağlıdır. Ayırıcı tanısında bir çok durum vardır ve tanımlanmış standart bir yönetimi yoktur. Yazarlar, laparoskopik yaklaşımdan cerrahi olmayan tıbbi seçeneklere (çünkü HKL'den kaynaklanan kanama genellikle kendi kendini sınırlamaktadır) kadar ayırıcı tanı ve tedavide kullanılan modalitelerin detaylı bir karşılaştırmasını yapmaktalar. Yazarlar, literatür verilerine homojenlik vermeye çalışarak, HKL gelişimiyle ilgili tüm verileri gözden geçirdiler. Yazarlar literatür verilerini analiz ettiler ve tıbbi yaklaşımı birçok alt gruba ayırdılar. "Bekle ve gör" tutumu, destekleyici tedaviler (antifibrinolitik, analjezikler, sıvı infüzyonu, transfüzyonlar ve antibiyotik profilaksisi) kullanarak gereksiz laparoskopik cerrahiyi önler. Cerrahi tedavi laparoskopik yöntemle luteektomi, yumurtalığın kama şeklindeki eksizyonu veya ooferektomi şeklinde olmalıdır. Doğurganlığı korumak, özellikle kanama bozukluğu olan veya antikoagülan tedavi alan hastalarda önemlidir; bu nedenle yumurtlamayı önlemek ve başka HKL ataklarını önlemek için östro-progestiniklere veya GnRH analoglarına ihtiyaç vardır. Bu derleme, hekimlere HKL'nin erken teşhisini koymada, gereksiz cerrahiden kaçınmada ve en etkili tedaviyi kullanmada yardımcı olacaktır.
Anahtar Kelimeler: Korpus luteum, yumurtalık kisti, ektopik gebelik, laparoskopi

Introduction

Ovulation is a physiologic monthly event and may be rarely complicated by rupture of the corpus luteum (CL), which occurs at all stages of a woman's reproductive life. The CL is formed during the luteal phase of the ovarian cycle. Spontaneous but self-limiting bleeding fills the central cavity, and when bleeding is excessive, the CL enlarges and forms a hemorrhagic CL cyst, which may rupture. Bleeding from a ruptured CL can vary from mild hemorrhage to massive hemoperitoneum, leading the patient to shock and subsequent emergency surgery. Hemorrhagic corpus luteum (HCL) rupture and bleeding are often triggered by exercise, coitus, trauma or a pelvic examination. Clinical symptoms are mainly due to peritoneal irritation by the blood effusion. The differential

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diagnosis is extensive and includes ectopic pregnancy, adnexal torsion, neoplasm, and pelvic inflammatory disease (PID)[1,2]. The actual incidence of HCL is unknown because it is often asymptomatic and escapes the attention of physicians. Hallatt et al.[1] reported a higher prevalence in young women. Most ruptures occur during the secretory phase of the cycle. HCL is one of the differential diagnoses of acute abdomen in women of reproductive age. Although it can occur at any time of life, it is likely to develop in the early period after menarche. A significant correlation between coitus and rupture of the CL was described by Aggarwal et al.[3], showing a traumatic etiology in 16 of 26 patients (61%). An increased frequency of HCL during pregnancy was also observed, with increased risk of spontaneous miscarriage[1].

The authors reviewed the clinical findings of HCL and elaborated a comparison of differential diagnosis and therapeutic modalities from the laparoscopic approach to nonsurgical, medical options.

In this investigation, we reviewed all data implicated with HCL development, such as age, race, heritage, reproductive factors, sex hormone, obesity, diet, smoking, physical activity, stress, talc use, and environmental and other factors. To perform the clinical research, the authors consulted the following scientific databases: PubMed (1966-2019), the Cochrane Library, Google Scholar (1966-2019), EMBASE (1974-2019), Science citation index (1974-2019), The China Journal Full Text Database (1994-2019), Chinese Scientific Journals Full Text Database (1989-2019), Chinese Biomedical Literature Database (1978-2019), and the WANFANG database (1980-2019).

The following key terms were used to access the records: ***corpus luteum***, ***corpus luteum rupture***, ***bleeding***, ***cyst***, ***pregnancy***, ***hemorrhagic corpus luteum***, ***corpus luteum rupture***. The authors reviewed all articles relating to the CL and problems related to the CL, such as the onset and complications, the bleeding caused by the CL, and the pharmacologic and surgical treatment. Randomized controlled studies were used when available; otherwise, literature that was the most relevant to the topic was used at the authors' discretion. Peer-reviewed articles regarding the CL and hemorrhage, sorted by relevance, were included for the aims of this work. Additional articles were identified from the references of retrieved papers. The aim of this extensive review was to provide information about the clinical and surgical data implicated with the development of HCL and its treatment modalities, trying to give homogeneity to literature data.

Pathophysiology and Risk Factors

According to Novak and Woodruff, bleeding does not normally occur from the stigma because it fills with fibrin. Ovulation is followed by a stage of proliferation or hyperemia, consisting of follicular collapse and luteinization of the granulosa layer, which is devoid of blood vessels. The lumen of the CL still contains no blood. The stage of vascularization then occurs; the granulosa layer is penetrated by blood vessels that fill the cavity of CL with blood[4]. If the CL hematoma ruptures, intraperitoneal hemorrhage may occur, especially if a woman's clotting mechanisms are depressed by anticoagulant therapy or congenital bleeding disorders. In this study, we focused exactly on this type of HCL hemorrhagic cysts.

Dextro-preponderance

Many studies observed a higher prevalence of HCL on the right ovary.

Aggarwal et al.[3] reported a dextro-preponderance of HCL in 20 of 26 patients (76.9%).

The study of Tang et al.[5] reported a right prevalence of 81.25%, and proposed that the right preponderance was the result of a different venous architecture causing higher venous pressure in the right ovary. Ho et al.[6] observed a rupture of CL in the right ovary in 60 of 91 patients (65.9%).

According to some authors, the presence of the rectosigmoid colon protects the left ovary from trauma, especially during sexual intercourses[1,3,6].

Pain in the right iliac fossa may mimic acute appendicitis, and the same symptoms related to the left quadrant might be underestimated in many patients, and this may contribute to the increased right prevalence of HCL[6].

Fukuda et al.[7] reported a greater ovulatory activity by the right ovary (~ 55%) than the left ovary during its entire reproductive age.

Bleeding Disorders and Anticoagulant Therapy

Patients with bleeding disorders have a greater risk of extensive hemoperitoneum than patients with normal coagulation function, and the former often require surgery to stop the bleeding.

Many cases of hemoperitoneum have been reported in patients with von Willebrand disease type 1, 2A, 3[8-14], patients with afibrinogenemia[15-17], patients with Glanzmann's thrombasthenia, patients with hemophilia A[18], hemophilia B, deficiency of factor X and factor XIII, and in patients receiving anticoagulant therapy for antiphospholipid antibody syndrome[19-22].

Hemorrhage from a ruptured CL cyst should be considered in any woman of reproductive age who is undergoing anticoagulant therapy because it is a potentially life-threatening complication. When ovarian enlargement and hemorrhagic effusion are detected, anticoagulant therapy should be stopped.

In women with known bleeding disorders or receiving anticoagulant therapy, HCL rupture should always be suspected in the presence of lower quadrant abdominal pain[2,23].

Complete coagulation screening is essential for the early identification of patients with bleeding disorders; anamnesis, anticoagulant therapy, and family history can provide important information.

These patients show a higher risk of recurrent HCL and ovulation should be suppressed with either a low-dose oral contraceptive pill (OC), progesterone-only agents or gonadotropin-releasing hormone analogs.

Clinical Aspects

Rupture of CL may be asymptomatic or associated with the sudden onset of lower abdominal pain. The pain often begins during strenuous physical activity, such as exercise or sexual intercourse, often lasting less than 24 hours.

Symptoms start in a third of patients with intermittent cramps preceding the acute pain, due to hemoperitoneum resulting from the rupture[1].

The cramps are caused by the luteal cavity distension due to the intracystic bleeding and the pain can range from a diffuse tenderness to acute abdomen when the rupture and the consequent hemoperitoneum occurs; even a small peritoneal effusion is large enough to cause real acute abdomen. Other symptoms may include nausea and vomiting caused by visceral reaction due to peritoneal irritation, vaginal bleeding, weakness, hypotension, syncope, and cardiovascular collapse. Visceral pain can also be related to emotional signs such as marked anxiety and autonomic signs such as pallor, sweating, nausea, vomiting, bradycardia or tachycardia. These signs further amplify the symptoms caused by bleeding.

Barel et al.[24] reported abdominal pain as a prevalent and constant symptom in all patients; 10.7% also had fever, 13% had nausea and vomiting, and 4% showed urinary disorders.

It is worth mentioning that it is not always clinically possible to differentiate hemorrhagic cyst and ruptured hemorrhagic cyst. In many cases of RCL, patients remain hemodynamically stable, and a moderate amount of free fluid in the abdominal cavity could be a normal finding in the postovulatory period. For this reason, we discuss both conditions naming it "HCL cyst" in the article.

Diagnosis

A physical examination of the abdomen and vagina is critical in the first evaluation of the patient. Accurate diagnosis depends on the clinical presentation, the results of tests, and the index of suspicion. A negative pregnancy test is important to exclude ruptured ectopic pregnancy.

Some diagnostic tests are necessary. Primarily, diagnosis requires an ultrasound (US) examination (very useful to inspect the CL and the abdominal effusion), a pregnancy test, a complete blood count, blood clotting tests, and an evaluation of inflammatory markers.

Laboratory Tests

For a patient's hemostasis evaluation, the following parameters should be evaluated:

• Prothrombin time (PT): This is used to assess the extrinsic pathway of coagulation. PT is longer in cases of factor VII, X, V, II, and fibrinogen deficiency, and it is essential to evaluate the hepatic synthesis of coagulation factors and vitamin K status, as well as for monitoring anticoagulant therapy.

• Activated partial thromboplastin time (aPTT): The aPTT is used to evaluate factors of the intrinsic and common pathway of coagulation and to monitor therapy with unfractionated heparin; it could be longer in cases of a shortage of one of the intrinsic pathway factors, or in the presence of antiphospholipid syndrome.

• Fibrinogen: This is reduced in case of liver diseases, CID, and massive transfusions, and is increased during inflammation.

• White blood cell (WBC): Barel et al.[24] observed that elevated WBC (11,000 per mL) was related to the severity of the clinical presentation and its value regresses with resolution[25].

• Hemoglobin (Hgb) and hematocrit (Hct): These tend to decrease progressively and proportionally to the amount of peritoneal effusion. Monitoring the changes Hgb levels is essential to assess the development of blood loss to evaluate the possibility for emergency surgery.

• Platelets: generally indicated to investigate the presence of possible thrombocytopenia or thrombocytopathy in patients with an HCL, without a history of bleeding disorders or anticoagulant therapy[23].

The human chorionic gonadotropin free beta-subunit (beta-hCG) is detectable in blood through radioimmunoassay after four weeks of amenorrhea, and in the urine from the sixth and seventh weeks of gestation age. Beta-hCG is essential to identify pregnant patients and in making a differential diagnosis with extrauterine pregnancy or intrauterine gestation.

Imaging-Ultrasound

US technology and in particular the use of transvaginal imaging has a key-role in the HCL diagnosis[26,27].

The sonographic appearance of a hemorrhagic ovarian cyst can be different in size, thickness of the cyst wall, and internal echo pattern depending on the formation and lysis of the clot[28].

Usually, HCL appears as a round ovarian mass with a mean diameter of 3.0-3.5 cm, with well-defined, regular and thin walls (Figure 1)[29].

Primarily, the clot forms like a fine fibrin network in the central cavity; subsequently, the coagulum in the cavity forms an organized reticular pattern[29,30]. After the luteo-lysis, the CL becomes "corpus albicans," which is not always visible with ultrasound.

The fibrin in the central clot of the CL appears like solid septa and interdigitations forming a "complex mass", entering into the differential diagnosis from ovarian neoplasm[29-32].

For this reason, Yoffe et al.[33] called HCL "the great imitator", although there is always one posterior acoustic enhancement that allows to distinguish it from a solid lesion[30].

Ding et al.[34] observed 104 cases of hemorrhagic ovarian cyst, describing four different US patterns: 20.2% showed a diffused dense echo pattern, 24.0% showed a mixed pattern, 28.8% showed a sponge-like pattern and 27.0% showed a cystic pattern. They also observed a ring blood flow (ring of fire) with high velocity and low resistance without internal blood flow in 40% of cases (Figure 2).

Several studies have evaluated the sonographic and Doppler characteristics of the CL[35-38] and the blood flow characteristics

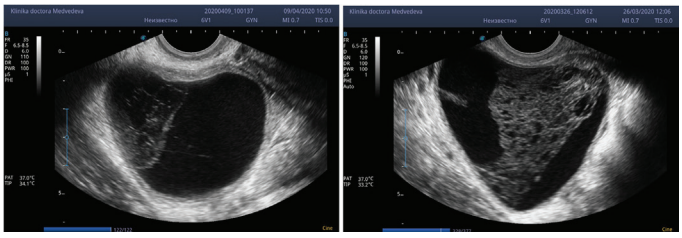

Figure 1. Typical sonographic features of a corpus luteum cyst with hemorrhagic content

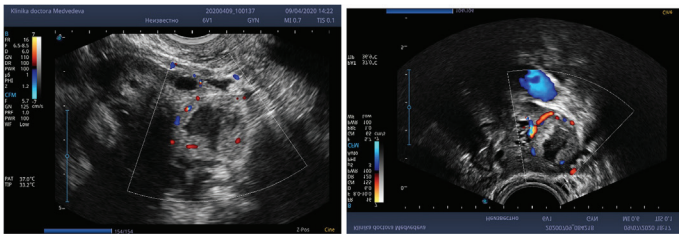

Figure 2. Doppler features of CLC (ring of fire)

have been well defined.

Tamura et al.[39] investigated the changes in CL blood flow during the luteal phase and early pregnancy. The relatively high resistance index (RI) during the late follicular phase declined with progression towards the luteal phase. By the mid-luteal phase, the RI was low, indicating a high blood flow to the CL. There was an increase in RI and therefore a reduction in the blood flow on regression of the CL. In women with luteal phase defects, the RI was significantly higher, indicating a decrease in blood flow. During pregnancy, the RI remains at the low mid-luteal phase level for the first 7-8 weeks and then increases once the CL regresses.

An abdominal or transvaginal US can also show hemorrhagic effusion into the abdominal cavity, especially at the lowest points such as the pouch of Douglas, the vesico-uterine pouch, and the iliac fossae. Hemorrhagic effusion can also be observed in the Morrison pouch[6] and the paracolic lodges. The amount of effusion can vary from minimal to massive bleeding.

Examination of endometrium reveals a secretory pattern during the luteal phase and indicates an active production of progesterone.

Although ultrasonography is superior to computed tomography (CT) for making the diagnosis, the appearance of hemorrhagic cysts on CT scans depends on the age of the clot: blood from an acute hemorrhage has a high attenuation value, whereas blood from a previous hemorrhage has an attenuation value approaching that of water. In an acute setting, CT typically demonstrates a cystic adnexal mass with areas of high attenuation in the intramural and intracystic sites. Hemoperitoneum may also be present, with high-attenuation clot and blood accumulating in the pelvis. The pretreatment CT scan for ruptured corpus luteal cysts can suggest the necessity of surgical treatment based on image findings[40,41].

Differential Diagnosis

There are several pathologies that need a differential diagnosis with HCL. The pelvic pain is typical of many gynecologic disorders, such as ectopic pregnancy, PID, ovarian torsion, and several non-gynecologic diseases such as appendicitis, gastroenteritis, cystitis, and other urinary tract disorders[42-44]. Recently, a case of ruptured hemorrhagic ovarian cyst presenting an incarcerated inguinal hernia in an adult female was reported[45].

The US differential diagnosis between some types of adnexal masses and complex malignant lesions is necessary[29]. Bleeding may be generally due to the rupture of an ectopic pregnancy, an infiltrating neoplastic disease, vascular diseases and traumas. Clinical history and anamnesis of patients are very important for a differential diagnosis. In the event of a positive pregnancy test, the physician must investigate other nonspecific clinical findings of an ectopic pregnancy (e.g. lower abdominal pain, cervical motion tenderness, a palpable adnexal mass and uterine spotting). The earliest appearance of symptoms generally occurs in the sixth week after the last period. Peritoneal signs are indicative of intraperitoneal blood collection. Usually, the pain is alternating and spasmodic, followed by intervals free of symptoms[46].

In most extrauterine pregnancies beta-hCG production is lower, the endometrium response is not stable, and spotting is common[46-48]. In 70% of ectopic pregnancies, the beta-hCG levels rise more slowly, reaching a plateau and even showing a decrease in serum levels. An abnormal beta-hCG pattern is highly suggestive of an ectopic gestation or a no longer intact gestation. Besides the unconventional rise in beta-hCG levels compared with normal pregnancies, ectopic pregnancy can be differentiated from a spontaneous abortion by a slower decrease in serum titer[49-52]. In a normally developing pregnancy, beta-hCG levels double every 1.5 days in the first 5 weeks of a regular gestation.

With a serum beta-hCG level of 1,500 mIU/mL, an intrauterine chorionic sac can be detected by transvaginal scan (TVS) presenting a double echogenic ring around a hypoechoic gestational sac called “comet sign”. Conversely, in patients with beta-hCG of 1,500 mIU/mL and more, an empty uterine cavity visualized by TVS with specifications as above can be taken as indirect proof of ectopic pregnancy.

The “tubal Ring” is a classic sonographic sign of ectopic pregnancy, represented by a hyperechogenic thick wall with anechoic content; only rarely it is possible to detect the yolk sac, which is often confused for a normal CL cyst[53,54]. Another significant sign of ectopic pregnancy is the “ring of fire”. Observed using the color Doppler, which is caused by vascularization of the placental blood flow of ectopic pregnancy. This sign, however, is similar to the one observed in the CL for the vascularization of the theca cells[55]. Therefore, these signs are not clear enough to differentiate an ectopic pregnancy from a CL. Frates et al.[54] observed that the hyperechoic walls of the

"tubal ring" of ectopic pregnancy reflected the hyperechoic area observed in intrauterine pregnancies at the initial stage, formed by the fusion of the trophoblast and the decidua. In several patients with ectopic pregnancy, the "tubal ring" has higher echogenicity than ovarian parenchyma and endometrium, and the walls of the CL show the same echogenicity, often appearing isoechoic or more often hypoechoic.

Visualization of the characteristic CL blood flow may aid in the diagnosis of ectopic pregnancy because about 85% of all ectopic pregnancies are found on the same side as the CL. This explains why in the majority of cases with proven ectopic pregnancy, luteal flow is detected ipsilaterally of the ectopic pregnancy. Luteal color or power Doppler flow may be used as a guide while searching for an ectopic pregnancy and could be called the "lighthouse effect" of CL, which directs the investigator to the color Doppler signals of the ectopic pregnancy[53-58].

Vidakovic et al.[59] reported a case of a ruptured CL cyst in early pregnancy with the authors suspecting an ectopic pregnancy, but after surgery, which confirmed HCL, a normal intrauterine pregnancy was found.

Torsion of the ovarian pedicle is also one of the most common complications of ovarian neoformations.

It usually occurs suddenly and can also affect normal adnexa (frequently the right adnexa). Venous stasis caused by torsion of the pedicle, like in the case of pelvic varices with the increase of vein blood pressure, can result in ovarian edema associated with HCL, as shown by Beyth et al.[60].

The prevalent symptom is abdominal pain as in the HCL and the abdomen appears slightly treatable and tense. Color Doppler US is diagnostic if it shows the reduction or absence of blood flow.

Cysts presenting rapid growth may break due to abnormal vascularity in some parts of the wall.

If the cyst contains blood, differential diagnosis with rupture of HCL becomes particularly difficult. Endometriomas may increase in size in every menstrual cycle and rarely break with acute abdomen and spreading content, with the dissemination of endometrial tissue into the peritoneal cavity. US is the first-line imaging technique for diagnosis. Endometriomas can have a variety of US appearances. The "classic" endometriosis cyst has been described as a homogeneous, hypoechoic focal lesion within the ovary; the majority of the endometriomas show diffuse low-level internal echoes, multilocularity and hyperechoic foci in the wall[61,62].

The differential diagnosis between HCL and endometriosis cysts, as well as the US appearance, is based on the US revaluation after the menstrual event, or at 4-6 weeks[42].

The disappearance or regression of a previously observed cyst is indicative of CL, while the persistence of the cyst is indicative of endometriosis cysts.

In cases of PID, patients show inflammation signs such as fever, severe pain, and increased WBCs and other inflammatory markers; these signs are found only rarely in case of HCL.

In cases of PID, US examination of tubo-ovarian abscesses will usually show a complex adnexal structure, with increased vascular flow compared with endometriosis cysts, with thick walls and internal pus-like echoes with cellular debris. During transvaginal examinations, patients may exhibit tenderness over the area of the fluid collection[61].

Classically, appendicitis diagnosis is based on symptoms, findings in a physical examination such as positive Blumberg sign, symptoms of acute abdomen, and severe leukocytosis. Acute abdomen and leukocytosis may both occur in case of HCL, but more often it is just a mild reaction without peritoneal muscle contracture and a modest increase in leukocytes levels[24]. US often shows an increase of the wall thickness and appendix edema, both typical of an inflammatory state.

If diagnostic doubt persists and the clinical symptoms appear in evolution, further imaging exams (e.g. CT and MRI) before performing a diagnostic laparoscopy are indicated.

Corpus Luteum of Pregnancy

The CL of pregnancy is simply the persistence of a CL that accompanies conception. It is most prominent early in pregnancy and is critical to sustaining gestation in the first 8 weeks when the maximum progesterone secretion occurs. As pregnancy evolves, the CL gradually regresses and plays a negligible role in the final two trimesters.

With routine ultrasonographic examination during the first trimester, the discovery of an ovarian cyst has become relatively common at the beginning of pregnancy.

Most unilocular and anechoic ovarian cysts with thin borders during the first trimester are CL cysts. They are not generally present after the end of the first trimester. Except in case of complications, surgery should be avoided. The complications of these cysts are represented mainly by torsion, intracystic bleeding, and rupture. Adnexal torsion is frequently associated with ovarian stimulation for ***in vitro*** fertilization or with ovarian masses, mainly of functional origin. Operative laparoscopy has become increasingly common in pregnant women over the past decade.

Emergency surgery during the first trimester for complications of an ovarian cyst, especially before the ninth week of amenorrhea, is associated with a high rate of abortion. In the second part of pregnancy, fetal morbidity with prematurity caused by emergency surgery is considerable. The ideal period for scheduled surgery is probably the beginning of the second trimester when the abortion rate is minimized[63].

A primary ovarian pregnancy is usually confused with tubal pregnancy, ruptured CL cyst, HCL, and ruptured endometrioma. This case presents the clinical and the histologic findings of a ruptured ovarian pregnancy, along with a ruptured CL cyst in the contralateral ovary.

Advances in laparoscopic technology and surgical techniques have overcome the technical difficulty of an enlarged gravid uterus[64].

It certainly seems that progesterone may still play an important role postoperatively in the first trimester if surgery involves the adnexa and/or CL. However, by the seventh week, the placenta takes over the role of producing progesterone to support the pregnancy.

Ruptured CL cyst of pregnancy with massive hemoperitoneum is a rare but life-threatening disorder that can occur suddenly. Ovarian conservative treatment should be laparoscopic if the patient's condition permits it.

Therapy

Once the diagnosis of hemorrhagic CL with hemoperitoneum has been confirmed, it is necessary to establish a therapy. The decision about the treatment of CL includes the "wait-and-see" option, with a continuous follow-up of clinical, laboratory parameters, and US detection.

In most cases, keeping patients under observation and waiting for the remission of symptoms is enough. In rare cases, surgery may be required to stop bleeding because hemodynamic instability and deterioration of clinical status can occur. Surgical management should be laparoscopic.

An article published in 1984, in which 173 surgical cases were analyzed, it was concluded that in most cases a correct pre-surgical diagnosis allowed the avoidance of surgery[1]. Ho et al.[6] showed that the use of surgery was significantly higher (100%) in the 1980s compared with cases examined between January 2001 and December 2003 (81.3%); however, the clinical manifestations of HCL were similar with those observed in the 1980s.

Wait-and-see Attitude

With the improvement of diagnostic tools over time, physicians have increasingly opted for a wait-and-see approach. Most cases of ruptured CL cysts with moderate hemoperitoneum can be treated conservatively[1,65].

During the observation period, it is important to continuously monitor US pelvic changes, hematocrit, and the symptoms reported by the patient.

The acute pain often subsides within the first 24 hours, and failure to improve could be a sign of worsening. Thus, during the observation period, it is recommended to perform another US evaluation and repeat the patient's blood count, especially with signs of anemia (fainting, tachycardia, pallor, dyspnea, asthenia).

If Hgb values are stable or are maintained above 12 mg/dL, and if a US evaluation is compatible with the previous one, surgical treatment is not indicated.

If the symptoms disappear and US scans and Hgb values are unchanged, the patient can be discharged the same day with the recommendation of returning to the hospital immediately if pelvic pain or signs of anemia appear, or with the indication to be periodically followed by a general practitioner.

Pelvic US is also recommended after complete resolution of the clinical symptoms, preferably at the end of the following menstrual period.

Drug Therapy

During the observation periods, physicians may prescribe drug therapy and supportive care; this, however, does not improve the outcome of the disease.

Antifibrinolytic

Tranexamic acid is one of the most widely used drugs in this category. Tranexamic acid is a synthetic derivative of the amino acid lysine, which exerts its antifibrinolytic effect through the reversible blockade of lysine binding sites on plasminogen molecules. There are no clear indications for anti-fibrinolytic treatment, but we know from a few trials that systemic tranexamic acid administered at the outset for surgery reduces intraoperative blood loss, and if it is administered within 3 hours of any injury, it significantly decreases blood loss[64].

Analgesics

They are used on patients' request to relieve painful symptoms, but pain does not always recede after the administration of these drugs.

Supportive therapies

Liquid infusion is certainly a useful remedy in patients with HCL. They can be administrated to increase the circulating volume mass and prevent a drop blood pressure. Glucose-infusions can also be used because patients should be kept fasting in case of surgical treatment requirement.

Transfusions

Sometimes blood transfusion is necessary, especially when Hgb and Hct are significantly reduced.

In cases of patients with bleeding disorders undergoing anticoagulant therapy, administration of fresh frozen plasma and vitamin K may be useful to replenish the missing coagulation factors[23].

Antibiotic Prophylaxis

Antibiotic prophylaxis is given to prevent bacterial superinfection of the pelvic effusion, which could result in real septic peritonitis. Antibiotic prophylaxis is conducted with broad-spectrum antibiotics active against the most common germs responsible for peritonitis. Prescription of antibiotics is made on an individual basis because the literature is lacking any evidence of their use in case of infection absence.

Surgical Therapy

Surgical treatment was, in the middle of the last century, the first choice and consisted mainly of a laparotomy with oophorectomy. With the advent of laparoscopy, a minimally invasive approach is preferred to laparotomy, which however remains the first-choice method in the event of cardiovascular collapse[66,67].

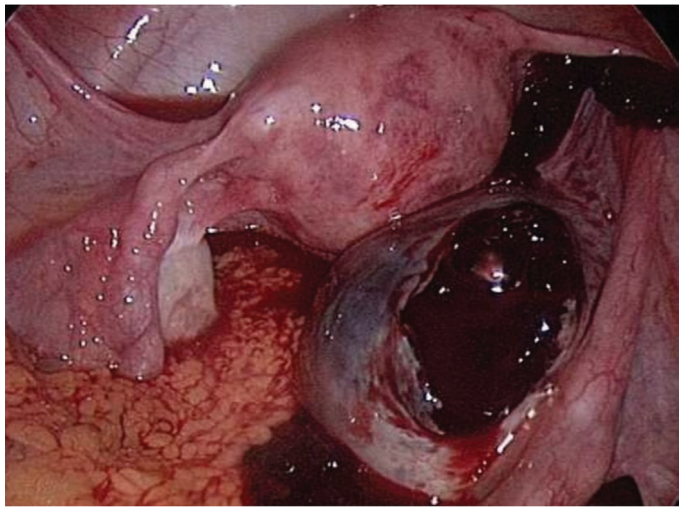

Figure 3. Laparoscopic view of ruptured CLC

Laparoscopy and Laparotomy

Laparoscopy is the preferred surgical approach because it results in less morbidity than laparotomy (Figure 3). It is well known that the laparoscopy has several advantages compared with laparotomy, which has been replaced almost completely in gynecology during the last years[65].

The first and most important advantage of laparoscopy is the type of incision, which is minimally invasive compared with laparotomic transverse incisions.

The hospitalization of the patient is reduced with laparoscopy compared with laparotomy (55±8 vs 98±14 hours) and post-operative pain is significantly reduced[68].

Complications risks are lower in laparoscopy, which however is more operator dependent.

In the event of massive hemoperitoneum, autologous blood transfusions from blood recovered from the peritoneal cavity should also be considered, even with the laparoscopic technique[69].

A limitation of the laparoscopic technique is the size of the cyst: if it exceeds the diameter size of 6-7 cm the indication to laparoscopy is limited to the surgeons' experience (this event is however quite rare in cases of HCL).

During laparoscopy, three kinds of surgical options can be used:

• Cystectomy or enucleation of ovarian cysts (luteumectomy): The technique is preferable because it allows the preservation of ovarian function.

• Ovarian wedge-shaped excision.

• Oophorectomy or ovariectomy: this was the preferred technique in the past and resulted in the total loss of the ovary, often accompanied by loss of the ipsilateral fallopian tube.

Women with Congenital or Acquired Bleeding Disorders

Women receiving anticoagulant therapy or with congenital disorders of the coagulation system have a higher risk of ruptured CL cysts. Surgical treatment is the traditional approach in this category of patients. There is still not enough evidence to support a surgical or expectant approach. According to some published case series, conservative treatment is the dominant trend in carefully selected patients with coagulopathies[70].

In summary, the observational approach in hemodynamically stable patients could be the first choice option in most cases. There are no data comparing these two strategies in this patient population, but if there are no concerns about ongoing brisk bleeding or infection or malignancy, the risks of surgery are not warranted. In cases of continuing bleeding or the decision of a patient to choose active management, a laparoscopic approach should be suggested with ovary-sparing surgery, using minimal energy.

Outcomes

There are scant data regarding the outcomes of ruptured ovarian cysts. Available studies include:

• In series of women with a ruptured ovarian cyst and hematoperitoneum, 15 of 78 were managed surgically[65]. Patients who underwent surgery had a more rapid Hgb decrease over 4 hours (1.7 vs 1.3 g/dL) and had higher transfusion rates than those managed conservatively (53 vs 11%).

• In another series of women with RCL, 58 of 70 women were managed with surgery and the remainder was managed with observation the study did not give rates of surgical complications, transfusions or recurrence[66].

Prevention of Recurrence

There are no known methods to prevent rupture of an existing ovarian cyst, except for surgical drainage or removal of the cyst. Patients with bleeding disorders or undergoing anticoagulant therapy have a higher risk of recurrence. Regular follow-up can minimize the risks. These patients often undergo surgery, with a higher risk of decreased ovarian function and adhesion formation, which consequently contributes to reduced fertility rates.

Thus, the possibility of preserving a future pregnancy is essential and patients need drugs such as estro-progestinics or GnRH analogues to prevent ovulation. Numerous studies have investigated the effects of OCs on follicular cyst development and ovulation. In general, current OCs resulted in the development of fewer follicular and correspondingly lutein cysts[23].

Conclusion

This review focuses on the pathophysiology, clinical presentation, diagnosis, and treatment options of HCL, also in patients with bleeding disorders or pregnancy complicated by CL cysts.

Rupture of CL is a common occurrence in women of reproductive age. Management is based upon patient characteristics, including the severity of symptoms, whether there is hemodynamic instability[1]. Currently, US is considered the gold standard technique in conjunction with clinical and laboratory findings for the diagnosis of HCL. HCL is clinically known to simulate several medical, surgical, and gynecologic

conditions that cause acute abdomen[42]. The most important is ectopic pregnancy.

The "tubal ring" is a classic sonographic sign of ectopic pregnancy[53,54].

Beta-hCG is essential for identifying pregnant patients and making a differential diagnosis with extrauterine pregnancy and intrauterine gestation or CL.

Observation is an adequate option in hemodynamically stable patients, without severe abdominal pain and in the presence of a small amount of pelvic fluid demonstrated by US. When a large amount of fluid is observed and/or in the presence of severe abdominal pain laparoscopy should be performed on admission. Direct laparotomy is mandatory in cases of circulatory collapse.

The decision on the treatment of CL includes the "wait-and-see" option with a continuous follow-up of clinical, laboratory parameters, and US detection.

During observation periods, drug therapy and supportive care are suggested. A careful pre-surgical diagnosis can often avoid the need for surgery.

Surgery may be rarely required to stop bleeding because hemodynamic instability and deterioration of clinical status can occur.

Patients with bleeding disorders or undergoing anticoagulant therapy have a higher risk of recurrence and often undergo surgery, with a higher risk of decreased ovarian function and fertility rates[23].

This article provides an overview of HCL, with a focus on helping physicians to identify the clinical signs and sonographic features early, to quickly diagnose this condition, to choose the appropriate treatment for their patient, and to prevent recurrent episodes.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.T., Concept: A.T., M.V.M., Design: A.T., Data Collection or Processing: A.T., Analysis or Interpretation: M.V.M., Literature Search: M.V.M., A.M., S.G., Writing: M.V.M.

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Review / Derleme

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Adenoid cystic carcinoma of Bartholin's gland diagnosed after lung lobectomy: Review of the literature and a case presentation

Segmental akciğer lobektomi sonrası rastlanan adenoid kistik Bartholin kanseri: Literatür değerlendirilmesi ve olgu takdimi

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Abstract

Bartholin's gland was first identified in human female in 1675 by Caspar Bartholin. The Bartholin gland is composed of several epithelial types: The body is mucinous acini, the duct is predominantly transitional epithelium, and the orifice is the squamous epithelium. Primary carcinoma of the bartholin's gland is an uncommon neoplasm. Adenoid cystic carcinoma (ACC) of bartholin gland carcinom is a rare variant of bartholin gland carcinoma, comprising 15% of all bartholin gland malignancies. ACC of the Bartholin's gland is characterized by slow growth so recurrence and distant metastases can take a long period. So distant metastasis has been found in only a few cases to the lungs, liver, bone and brain. Here, we present the case of Bartholin's gland ACC after four years follow up and presented with a lung metastasis.
Keywords: Adenoid cystic carcinoma of the Bartholin's gland, vulvar cancer, Bartholin gland carsinom

Öz

Bartholin bezi 1675 yılında Caspar Bartholin tarafından kadın genital anatomisinde tanımlanmıştır. Bartholin bezi çeşitli epitel tipleri ile kaplıdır; gövdesi müsinöz, kanal transisyonel ve orifis skuamöz epitelle kaplıdır. Primer bartholin kanseri nadir görülmektedir. Bartholin gland kanserinin adenoid kistik karsinom ise çok nadir görülmekte ve bartholin kanserlerin %15'ini oluşturmaktadır. Literatürde adenoid kistik karsinomlu 90'dan az hasta tariflenmektedir ve yavaş büyüme paterni nedeniyle rekürrens ve uzak metastaz uzun vakit alabilmektedir. Dolayısıyla akciğere, karaciğere ve kemiğe uzak metastaz çok az hastada rastlanmaktadır. Biz 4 yıllık hastalıksız takip sonrası akciğer lobektomisinde saptanan adenoid kistik bartholin kanseri olgusunu sunmaktayız.
Anahtar Kelimeler: Adenoid kistik Bartholin karsinomu, vulvar kanser, Bartholin bezi karsinomu

Introduction

The Bartholin gland was first identified in the human female body in 1675 by Caspar Bartholin[1]. The main function of the Bartholin gland is to secrete mucus to provide vaginal and vulvar lubrication. Each Bartholin gland is approximately 0.5 cm in size and drains mucous into a duct 2.5 cm long. The ducts open onto the vulvar vestibule at the four and eight o'clock positions on each side of the vaginal orifice. The Bartholin gland has different epithelial types: the body is mucinous acini, the duct is transitional epithelium, and the orifice is squamous epithelium[2]. Primary carcinoma of the Bartholin gland is an uncommon neoplasm. Bartholin gland carcinoma (BGC) comprises approximately 0.1 to 5% of all vulvar carcinomas and <1% of female genital malignancies[3]. BGC has many histologic types: adenocarcinoma, squamous, adenosquamous, transitional cell carcinoma, and adenoid cystic carcinoma (ACC)[4]. Adenocarcinomas and squamous cell carcinomas each account for approximately 40% and adenosquamous carcinomas account for approximately 5% of BGCs[5]. ACC of BGC is a rare variant, comprising 15% of all Bartholin gland malignancies and the first documentation of ACC was identified by Klobin in 1864[6]. In the literature, fewer than 90 patients have been described. ACC of the Bartholin gland is characterized by slow growth, so recurrence and distant

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metastases can take a long period. Only in rare cases are distant metastasis seen in the lungs, liver, bone, and brain. We present a case of ACC in a woman who presented with lung metastasis after four years of follow-up.

Case Presentation

In September 2014, a 58-year-old gravidity 5 parity 2 menopausal women presented with a palpable mass and vulval pain of the left Bartholin's gland area. Under general anesthesia, the left Bartholin's gland was excised with a Bartholin gland cyst prediagnosis. A pathologic examination revealed an ACC of the Bartholin gland, the tumor continued at the positive edge of surgery with negative perineural invasion. The unexpected malignant lesion was diagnosed and a scientific study was conducted for metastatic disease. Chest, abdomen, and pelvis computed tomography (CT) scans showed no metastatic disease. Then the patient underwent hemivulvectomy with left inguinofemoral lymph node dissection. After surgery, the pathology result showed a tumor on one side of the surgical margin and positive perineural invasion. The inguinofemoral lymph nodes were collected, all of which were tumor-free. The patient received no adjuvant treatment. We followed up the patient regularly and who showed no recurrence over a 4-year period. Forty-nine months after the surgery, she had chest pain and cough symptoms. A thorax CT scan was performed, which showed a right upper lung lesion in diameter of 1.2*1.1 cm (Figure 1). In September 2018, the patient underwent a left pulmonary wedge resection. The diagnosis was clarified with pathologic results. It showed a lung metastasis of ACC with no tumor in lymph nodes and margins are negative (Figure 2). A pathology examination showed columns of cells arranged concentrically around gland-like spaces filled with eosinophilic periodic acid-Schiff-positive material. In addition, the examination showed the characteristic tumor proliferation in a cribriform pattern composed of nests, hence, the presence of ACC metastasis. Two years earlier, when the first ACC was diagnosed, it showed similar speciality and was clarified with immunohistochemical characteristics. Tumour cells were widely positive with SMA, CD117, and p63, and focally positive with CK7 and calponin (Figure 3). The patient is now disease-free with 56 months of follow-up and stable disease.

Discussion

In 1864, Klob was the first to describe BGC. It has various types such as adenocarcinoma, squamous, adenosquamous, transitional cell carcinoma, and ACC. Ten to fifteen percent of patients have ACC, which is histologically similar to adenocarcinoma of the salivary glands[7]. ACC of the Bartholin gland is extremely rare. Only 80 cases have been reported in the literature[8]. Common symptoms are a painless mass, pruritus, dyspareunia, burning sensation, vulvar pain, and abnormal bleeding. Initial misdiagnosis or delayed diagnosis may occur in up to 50% of patients, with incorrect diagnoses of Bartholin cysts or abscesses[7]. The most frequent symptoms is a progressive enlargement or swelling in the vulva; the patients often experience pain, which is probably due to tumor involvement of nerves[9]. In our case, the patient was misdiagnosed as having a Bartholin cyst and underwent Bartholin gland excision. The patient underwent reexcision and inguinofemoral lymphadenectomy because of incomplete surgery and margins with the tumor. The Bartholin gland was excised for the primary treatment. In women aged over 40 years diagnosed with Bartholin cysts, fine needle aspiration cytology is to exclude malignancy recommended[10]. The incidence of Bartholin gland tumors is highest among women in their 60s. The incidence of BGC in one series was 0.023 per 100,000 woman-years in premenopausal women and 0.114 per 100,000 woman-years in postmenopausal women[11]; however, ACC of the Bartholin gland shows a different age predisposition. ACC can be shown from the late 20s[8]. ACC of the Bartholin's gland

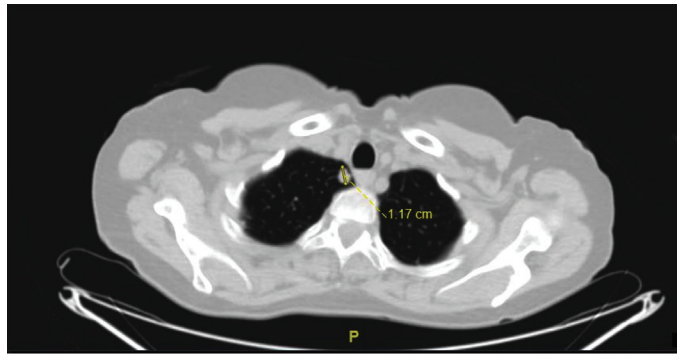

Figure 1. CT scan showed right upper lung lesion in diameter of 1,2*1,2 cm

CT: Computed tomography

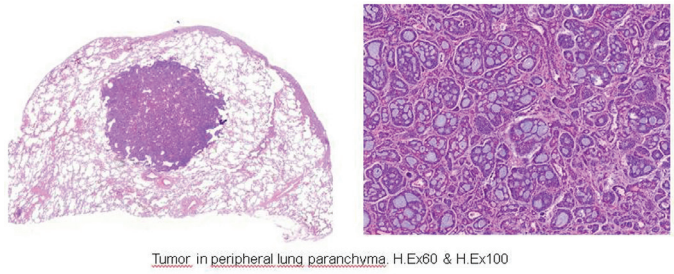

Figure 2. Tumor in peripheral lung paranchyma

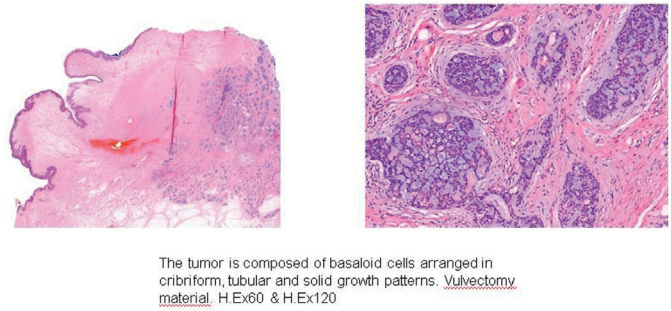

Figure 3. Tumor in vulvectomy material

shares classic histologic features with ACC of the salivary gland. The tumor is composed of uniform, small cells arranged in cords and nests with a cribriform pattern, variable-sized cysts filled with an amphophilic or eosinophilic acellular basement membrane-like material. The tumor must be located in an appropriate anatomic location. Most tumors have perineural invasion, which is thought to contribute to its high local recurrence rate[12]. ACC of the Bartholin gland is characterized by slow growth, local recurrence, and distant metastases by intravascular spread may occur over a long period[13]. Distant metastasis of ACC is not common, it is very rare in the lung, brain, liver, and bone. Alsan et al.[8] showed the most prevalent distant metastasis of ACC site was lungs, followed by liver, and rarely bone. Our patient had a distant metastasis after 4 years of follow-up.

Optimal surgical treatment for ACC of the Bartholin gland is not identified with guidelines. Radical vulvectomy ± inguinal lymphadenectomy and simple excision can both be performed. The most important point is to achieve a tumor-free margin. Yang et al.[14] showed 68.9% of patients who had a simple excision had recurrence rate. Patients who underwent radical vulvectomy had a 42.9% recurrence rate; resection margins in the radical vulvectomy group were positive in 30% of the patients. The positive margin rate was 48% in the simple excision group. Hsu et al.[15] presented two ACCs of Bartholin gland origin with lung metastasis; the first patient had a positive margin in pathologic examination and received postoperative adjuvant external beam radiotherapy, and distant metastasis (lung and bone) was found 42 months after the radiotherapy. The other patient who had tumor-free margins, had no adjuvant treatment. Fifty-nine months after the surgery she had lung metastases. Yoon et al.[16] presented 5 cases of ACC of the Bartholin gland. Two had lung metastases. In the first case, a 54-year-old woman had right-side radical local excision + ipsilateral inguinal lymph node dissection + adjuvan radiotherapy. Resection margins and perineural invasion were positive. She had lung metastasis twice 7 and 8 months later and underwent metastasectomy with subsequent chemotherapy (paclitaxel and carboplatin) for the first metastasis and metastasectomy only for the second metastasis. After 71 months' follow-up, she had stable disease. The second case was a 60-year-old woman who had left-side radical local excision and no adjuvant therapy. Resection margins and perineural invasion were positive. Local pelvic recurrence and distal metastases (lung) occurred in the patient, the local metastasis was removed. Lung metastases occurred 71.18 and 189 months after surgery and complete excision of the pulmonary lesion was not possible because of the number and size of the lesions. The patient had local excision and no adjuvant therapy. Resection margins and perineural invasion were positive. Local pelvic recurrence and distal metastases (lung) occurred in the patient, the local metastasis was removed. Lung metastases were at 71,179 and 189 months after surgery and complete excision of pulmonary lesion was not possible because the number and the size of lesions did not rise up.

After 224 months' follow-up she had progressive disease[16]. In our case, like Yoon et al.[16] cases, the patient had positive margins in pathologic examination, received no adjuvant treatment; she had lung metastases 49 months after surgery, and after 56 months follow-up, she has stable disease. When the distant metastasis was evaluated, it seemed to be related to margin positivity and perineural invasion. ACC of Bartholin's gland is a slow-growing tumor, long-term survival is excellent according to Copeland et al.[17] The 5-year progress-free interval is 47% and the 5-year survival rate is 71%. They are 38% and 50%, respectively, at 10 years, and 13% and 51% at 15 years. According to these results, it has been suggested to use 10- to 15-year-survival rates rather than 5-year survival rates for ACC of the Bartholin's gland. The patient of BG-ACC metastasis of Inguinal lymph node take place seldomly. However, if it occurs, it is ipsilateral to the primary tumor. In our case, there was no lymph node metastasis. In the literature, the effect of lymphadenectomy on survival and prognosis is controversial. There is little information on the treatment of metastasis and the management depends on the location. In literature, there are no data to support chemotherapy to prevent distant metastasis. If a distant metastasis exists, chemotherapy treatment alternatives such as chlorambucil-adriamycin, methotrexate-dactinomycin, cyclophosphamide-adriamycin-cisplatin or cyclophosphamide can be used[14]. Various treatment modalities, including radiotherapy, may produce tumor regression. Publications have emphasized poor outcomes in patients with positive tumor margins even when treated with radiotherapy[18]. A prospective randomized study can provide the most powerful evidence for deciding the optimal treatment. Due to the rarity of ACC of the Bartolin gland, we can obtain data from reviews and large series. ACC of Bartholin's gland is a rare, vulvar malignancy with unpredictable biologic behavior. Physicians have to suspect it in women aged over 40 years with persistent Bartholin's gland masses. There is no consensus on the treatment and the treatment modality must be tailored according to each patient. Radical local excision or radical vulvectomy ± lymphadenectomy seems to be the most suitable treatment. Positive surgical margins are a very important factor related to recurrence. Surgery, radiotherapy or chemotherapy regimens can be used for recurrence. Follow-up of ACC of the Bartholin gland has to be for a long period due its slow growth behavior.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.O., Concept: S.Ş.A., C.C.Ö., Design: S.Ş.A., Data Collection or Processing: S.Ş.A., Analysis or Interpretation: S.Ş.A., Literature Search: S.Ş.A., Writing: S.Ş.A.

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Case Report / Olgu Sunumu

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Severe ovarian hyperstimulation syndrome and gonadotropin-releasing hormone agonist trigger in patients with hypogonadotropic hypogonadism: A report of two cases

Hipogonadotropik hipogonadizm hastalarında ciddi ovaryan hiperstimülasyon sendromu ve gonadotropin releasing hormon agonistiyle trigger: İki olgu sunumu

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Abstract

Ovarian Hyperstimulation syndrome (OHSS) is a rare condition in patients with hypogonadotropic hypogonadism. Two patients with hypogonadotropic hypogonadism are reported, a rare case of severe OHSS and a case of prevented OHSS via gonadotropin-releasing hormone (GnRH) agonist trigger, respectively. The first case was a 31-year-old patient. *In vitro* fertilization (IVF) treatment was performed three times but the patient never developed OHSS. The first patient was diagnosed as having severe OHSS on the ninth day after the fresh embryo transfer. She stayed 66 days in hospital and 50.5 litres of fluid were aspirated from her abdomen. The second case was a 26-year-old and primary infertile patient. She had never undergone IVF treatment. The GnRH agonist stimulation test was performed before IVF treatment. After the ovarian stimulation, GnRH agonist trigger was given. Thirty-two oocytes were retrieved from the ovaries and OHSS did not occur. Although severe OHSS is rare, it can develop in patients hypogonadotropic hypogonadism. If a GnRH stimulation test is performed before ovarian stimulation, OHSS can be prevented because the test allows agonist triggering instead of hCG in hypogonadotropic hypogonadism.

Keywords: ART, freeze-all, GnRH agonist trigger, hypogonadotropic hypogonadism, OHSS

Öz

Over Hiperstimülasyon sendromu (OHSS) hipogonadotropik hipogonadizm hastalarında nadirdir. Burada ciddi OHSS olgusu ve gonadotropin salgılatıcı hormon (GnRH) agonist triggeriyle OHSS engellenmiş hipogonadotropik hipogonadizmi olan iki hasta sunulmuştur. İlk olgu 31 yaşında bir hastaydı. Üç kez *in vitro* fertilizasyon (IVF) tedavisi uygulanmış ancak olguda over hiperstimülasyon sendromu hiç gelişmemiş. Taze embriyo transferinden sonraki dokuzuncu günde şiddetli OHSS tanısı konuldu. Hastanede 66 gün kaldı ve batından 50,5 litre asit sıvısı aspire edildi. İkinci olgu 26 yaşında ve primer infertil hastaydı. Hiç IVF tedavisi görmemişti. IVF tedavisinden önce GnRH agonist stimülasyon testi uygulandı. Ovaryan stimülasyondan sonra GnRH agonist triggeri yapıldı. Toplanan 32 oosite rağmen OHSS meydana gelmemiştir. Hipogonadotropik hipogonadizm hastalarında şiddetli OHSS nadir olmakla birlikte gelişebilir. Eğer hipogonadotropik hipogonadizm hastalarında ovülasyon indüksiyonundan önce GnRH stimülasyon testi yapılırsa, hCG yerine GnRH agonist trigger yapılabilir ve OHSS önlenebilir.

Anahtar Kelimeler: ART, freze-all, GnRH agonist trigger, hypogonadotropic hypogonadism, OHSS

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Introduction

Hypogonadotropic hypogonadism (HH) is a puberty-delay and amenorrhea-causing disorder due to deficiency of gonadotropins and sex steroids in the circulation. Individuals with HH have defects either in the secretion of the gonadotropin-releasing hormone (GnRH) from hypothalamus or GnRH receptors in the hypophysis[1]. Ovaries are not stimulated depending on the deficiency of gonadotropins, thus, infertility follows in HH. Stimulation with exogen gonadotropins is crucial for fertility treatment because folliculogenesis and ovulation are defective in patients with HH. Human chorionic gonadotropin (hCG) injection is mandatory to mature oocytes in controlled ovarian stimulation because the luteinizing hormone (LH) peak does not exist in these patients[2].

Ovarian Hyperstimulation syndrome (OHSS) is the most serious, life-threatening, iatrogenic complication of assisted reproductive technology (ART) cycles. HCG is the major molecule that causes OHSS[3]. Therefore, to prevent and manage OHSS, GnRH agonists have been started to be used for final oocyte maturation. As a result, a segmented approach, including GnRH agonist triggering plus freezing of all embryos was recommended for the complete prevention of OHSS, especially for patients with an extreme ovarian response[4].

In general, OHSS is not common in patients with HH and hCG is the primary choice for oocyte maturation[5]. However, in some patients with HH, a hyper response may also occur and hCG triggering might be a life-threatening event in these patients. The exact pathology in patients with HH may be related to pituitary receptors or hypothalamus, and a patient with HH may not respond to GnRH agonists efficiently. Therefore, theoretically, GnRH agonists are not recommended to be used for triggering oocyte maturation in patients with HH[6].

In the following article, HH and agonist trigger will be discussed considering two patients with HH. In the first case, severe OHSS was seen, in the second, potential OHSS development was prevented using a GnRH agonist trigger.

Case Reports

The patients were treated in Novafertil *In vitro* Fertilization (IVF) Center, Konya, Turkey. Institutional review board approval was given (NIRB:2018-22) and written informed consent was obtained from the patients.

In 2013, a 31-year-old patient presented to our IVF center with symptoms of primary amenorrhea and secondary infertility. She was married for 11 years and had undergone a right salpingo-oophorectomy due to a dermoid cyst. Due to having HH, she underwent intrauterine insemination (IUI) treatment three times with hMG and IVF treatment three times. These trials resulted in one early period miscarriage and one healthy birth. The patient had never developed OHSS in any step of the treatments. To regulate her menstrual cycles, she took an estrogen (E2) and progesterone (P) combination. After the examination of the patient, other than a normal-sized uterus, small antral follicles were observed on the left ovarium, whereas the right ovarium was not monitored. In a previously taken hysterosalpingogram, the cavity and left tubal transition were normal; however, the right tuba was not monitored. Her body mass index (BMI) was 24 kg/m^2. The basal characteristics of the patient are illustrated in Table 1. Her blood analysis resulted in white blood cells (WBC): 8.2, platelets: 307, hemoglobin (Hgb): 13.6, and hematocrit (Hct): 40. On the third day of menstruation (E2+P replacement triggered menstrual cycle), ovarian stimulation was started with 300 IU hMG (Menogon 75 Ferring Turkey). When three dominant follicles reached 18 mm in diameter, the final stage of oocyte maturation was triggered using 5000 IU urinary hCG (Pregnyl amp, MSD, Turkey). To avoid OHSS, the patient was medicated with cabergoline (Dostinex Pfizer Turkey) for 6 days after the trigger day. Transvaginal ultrasound-guided aspiration was performed 36 hours later. Two embryos, each grade 1, were transferred and three grade 1 embryos were frozen on the third day following intracytoplasmic sperm injection (ICSI). IVF outcomes are shown in Table 2. On the embryo transfer (ET) day, her WBC, Hgb, and Hct values were at normal levels. On the 9th day following ET, she consulted our IVF center due to abdominal distention, swelling, and pain. Her ovaries were enlarged and abdominal fluid was observed on examination. Her blood analysis results were WBC: 22,400, Hct: 47.2, Hgb: 16.2, and beta-hCG: 61.7. Accordingly, she was accepted as having late OHSS and was hospitalized in a tertiary hospital. She stayed in hospital for 66 days. During this period, with 50,550 cc paracentesis fluid was aspirated; in total, 20% 100 cc human albumin and 0.9% NaCl was given 27 times, and hydroxyethyl starch was administered every day. The patient was discharged from hospital on the 13th week of the pregnancy and she gave birth to a healthy girl via cesarean section.

A 26-year-old woman consulted our IVF center with symptoms of primary amenorrhea and primary infertility. She was married for 3 years. In her history, it was remarkable that she responded insufficiently to clomiphene citrate and had no menstruation after medroxyprogesterone acetate was administered; therefore,

Table 1. Clinical characteristic of two patients with hypogonadotropic hypogonadism

	Case 1 (OHSS)	Case 2 (GnRH Ag trigger)
Age (Years)	31	26
BMI (kg/m^2)	24	30.1
Basal serum FSH (mIU/mL)	1	0.9
Basal serum LH (mIU/mL)	0.15	0.73
Basal serum Estradiol (pg/mL)	28.66	21
Basal serum TSH (mIU/mL)	2.82	2.43
Basal serum Prolactin (ng/mL)	6.5	7.1

OHSS: Ovarian Hyperstimulation syndrome, GnRH: Gonadotropin-releasing hormone, BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone

HMG was used until 225 units for controlled ovarian stimulation within an IUI plan. However, no response was received from the patient. When she was examined, it was determined that she had a small uterus and thin endometrium. Moreover, many small antral follicles were observed on both ovaries. Her basal characteristics are summarized in Table 1. Before starting treatment, a trial of GnRH analogue triggering was performed and 0.2 mg triptorelin (Gonapeptyl 0.1 Ferring, Turkey) was administered subcutaneously. After triggering, in the first and second hours, LH levels were 55 and 61 mIU/ML, respectively. Stimulation was started with 225 IU HMG (Menopur Ferring, Turkey) on the same day without waiting for menstruation. Follicle growth, as well as estradiol levels, were followed and gonadotropin doses were arranged according to the response. When at least three leading follicles (each >17 mm in diameter) were developed, oocyte maturation was triggered with 0.2 mg triptorelin. Thirty-two oocytes were retrieved 36 hours after the triptorelin injection. The IVF outcome of the patient is presented in Table 2. Following the first menstruation, she received hormone replacement therapy. One good quality embryo was transferred after the second menstruation using E2 hemihydrate (Estrofem 2 mg, Novanordisk Turkey) with a thawing protocol. This procedure resulted in pregnancy. At the time of writing, the patient was in her 13th week of pregnancy.

Table 2. *In vitro* fertilization outcomes of two patients with hypogonadotropic hypogonadism

	Case 1 (OHSS)	Case 2 (GnRH Ag trigger)
Days of stimulation (day)	13	21
Gonadotropin start dose (IU)	300	225
Total gonadotropin dose (IU)	3150	6625
Estradiol on trigger day (pg/mL)	5695	7797
No. of oocytes retrieved (n)	10	32
No. of oocytes that matured (n)	7	23
No. of fertilized oocytes (n)	6	19
No. of transferred embryo (s) (n)	2	1
No. of cryopreserved embryos (n)	3	12

OHSS: Ovarian Hyperstimulation syndrome, GnRH: Gonadotropin-releasing hormone

Discussion

In patients with HH, the aim of treatment is to achieve a single pregnancy through monofollicular development with stimulation and coitus or IUI. When ovulation induction and/or IUI fails or additional pathology (e.g. tubal obstruction or oligospermia) accompanies HH, treatment with ART is warranted. The first patient had severe OHSS, even though she had a single ovary. In the second case, response to stimulation was received over a long period and finally, an excessive ovarian response developed. However, the development of OHSS was prevented by performing an GnRH agonist stimulation test on the first day of treatment.

A fresh embryo was transferred to the first patient according to our IVF team's decision, social reasons, and low risk of OHSS. Although early OHSS development did not occur, it developed with the beginning of the pregnancy as late OHSS with a long recovery duration lasting until the 13th week of the pregnancy. Interestingly, she had a very serious OHSS clinic although she had a single small-sized ovary. Some 50.55 litres of acidic fluid was aspirated via repeated paracentesis. If the ET was cancelled, OHSS and its consequences would not occur. This case led us to reconsider the management of patients with HH with a hyper response to controlled ovarian stimulation.

Patients with HH are included in a heterogeneous population from the point of ovarian stimulation. Although the patients have the same clinical findings, their response to ovarian stimulation may be hyper, normal or poor. Both ovaries can be visualized as hypoplasic in ultrasonographic evaluation, hence, counting antral follicles is quite challenging. The prediction of the response to gonadotropins is challenging because the exact number of antral follicles cannot be identified and the levels of gonadotropins are below normal ranges[7]. AMH only reflects the growing follicular pool responsive to gonadotropins. Hence, conditions that cause a permanent or sustained interruption of gonadotropin release may lead to decreased AMH levels. Therefore, AMH is not a proper predictor of ovarian reserve in patients with HH, which may underestimate the exact ovarian reserve[8]. Determining the ovarian response before treatment is vital to provide acceptable rates of pregnancy with minimal adverse effects. Unfortunately, there is no reliable indicator for ovarian response in patients with HH except age[7].

A high dose and long duration are needed during stimulation, which increases the risk of OHSS because ovaries are dormant and doubt of developing poor-response. It was stated that the ovarian stimulation duration could range from 12 to 54 days[9]. On the other hand, despite the application of a high dose, OHSS development is quite rare in patients with HH[5,7,10-12]. If OHSS occurs, the clinical condition can be life-threatening, as in our first case.

Kuroda et al.[13] transferred a single frozen embryo with a freeze-all strategy to patients with HH to avoid the adverse effects of a fresh cycle on endometrial receptivity. They wanted to prevent a decreased response; therefore, the final oocyte was maturated with hCG, after considering the non-existence of OHSS in previous IVF cycles. They did not encounter any severe OHSS in their studies. Despite using the freeze-all strategy in his study, Kuroda et al.[13] did not employ GnRH, which is the main trigger agent. The major reason was to eliminate the possibility of a decreased response with agonist triggering.

The cause of HH can be detected by looking at LH and FSH peak levels, after a GnRH agonist stimulation test[14]. No cut-off values for LH or FSH peak have been indicated. An increase in

the peak equal or more than twofold is considered sufficient for maturation. This test would show a problem either with pituitary GnRH receptors or with GnRH production in the hypothalamus. If the condition is caused by pituitary GnRH receptors, LH and FSH levels will not increase, but if it is caused by GnRH, production gonadotropin levels will increase. Furthermore, even in women with GnRH receptor mutations, gonadotropin levels were detected to be increased like healthy controls in a previous study[15]. Women carrying GnRH receptor mutations were indicated to ovulate either spontaneously or in response to pulsatile GnRH administration via a portable pump[15].

In our second case, it was planned to start the stimulation with high-dose gonadotropin because her BMI was high and no response was obtained despite long-term gonadotropin use in the previous IUI cycle. The possibility of triggering with GnRH agonist was considered due to the risk of OHSS. GnRH agonist triggering was performed knowing that the patient would respond because the LH increment was observed on the first day of the GnRH stimulation test. Thus, OHSS development was prevented and a healthy pregnancy was acquired in the second case. If LH response cannot be obtained after a GnRH stimulation test, combined use of alternative OHSS prevention methods is suggested (3).

Early or late OHSS can develop in patients with HH. Severe OHSS can be prevented with GnRH agonist triggering instead of hCG triggering in patients with HH with a hyper response. The use of a GnRH stimulation test will be very helpful for the selection of patients who will respond to GnRH agonist triggering before controlled ovarian stimulation cycles in patients with HH.

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Ethics

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Authorship Contributions

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Letter to the Editor / Editöre Mektup

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Rectus abdominis muscle with different abdominal pathologies: A cite to myofascial trigger point

Farklı abdominal patolojilerle rektus abdominis kası: Miyofasyal tetik noktaya bir atıf

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Keywords: Rectus abdominis muscle, primary dysmenorrhea, myofascial pain syndrome

Anahtar Kelimeler: Rektus abdominis kası, primer dismenore, miyofasyal tetik nokta

Dear Editor,

I read the article, "Relation between uterine morphology and the severity of primary dysmenorrhea"[1] with interest. Although the etiologic factors in primary dysmenorrhea are covered in the article, the role of muscle structures in etiology is not mentied. In this article, we would like to discuss the rectus abdominis muscle and its myofascial trigger point (MTrP), which is another underdiagnosed cause of primary dysmenorrhea and abdominal wall pain.

The rectus abdominis muscle extends from the pubic symphysis and tubercle as a beam and ends at the anterolateral aspect of the xiphoid process and superiorly in three fragments of the costal cartilages of the ribs 5-7[2]. The rectus abdominis muscle facilitates flexion of the trunk and stabilizes the pelvis. Contributing to the increased abdominal pressure with other abdominal muscles, it also plays a role in micturition, defecation, vomiting, and childbirth. MTrPs of the rectus abdominis muscle have been associated with pathologies such as pain in the abdominal wall and primary dysmenorrhea[3,4].

Primary dysmenorrhea is a frequently encountered condition that reduces the quality of life of the patient and considerably impacts the economy of the healthcare system. Primary dysmenorrhea is a recurrent, cramping pain in the lower abdomen occurring during menstruation without any pelvic pathology[3]. Medical treatments, yoga, pilates and stretching exercises, massage techniques for soft tissues, and invasive approaches can be used to treat primary dysmenorrhea[3]. According to the literature, Gaubeca-Gilarranz et al.[3] administered placebo-controlled dry needle treatment for MTrP of the rectus abdominis muscle in patients with primary dysmenorrhea. Moreover, Huang and Liu[5] performed local anesthetic injection therapy to treat the MTrPs of the rectus abdominis and oblique muscles in patients with primary dysmenorrhea.

As observed by physicians, abdominal pain has been most frequently associated with intraabdominal pathologies. Therefore, numerous consultations and tests are required, and abdominal surgeries are occasionally performed, although the necessity thereof is debatable[1]. When patients cannot find a remedy for their pain, they visit various clinics for examinations, and certain patients are considered to have psychiatric disorders. In fact, MTrP of the rectus abdominis muscle causes abdominal wall pain. Muscolino EJ detected the MTrP of the rectus abdominis muscle in a patient with Crohn's disease who had a complex history of medical treatments and clinical courses[4]. A stretching exercise program for the relevant muscle was used to treat this patient.

The diagnosis of MTrP is under-recognized in clinical practice. On examination, the possibility of MTrP localization in each muscle should be considered. MTrPs of the rectus abdominis and related muscles can have different clinical manifestations. These patients should be thoroughly evaluated and appropriate treatment modalities should be used. Therefore, randomized

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controlled studies with a long-term follow-up are required in this field.

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