ISSN : 2149-9322
E-ISSN : 2149-9330

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

September 2017 Volume: 14 Issue: 3 www.tjoddergisi.org

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Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
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Printing at: Bizim Basım Limited Şirketi
Litros Yolu 2. Matbaacılar Sitesi ZD1 Topkapı, İstanbul, Turkey
Phone: +90 (212) 709 75 25
Printing Date: October 2017
ISSN: 2149-9322 **E-ISSN:** 2149-9330
International scientific journal published quarterly.

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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The journal is printed on acid-free paper.

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STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

STROBE statement-checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

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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.

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Original Research	250 words	5,500 words (~22 pages)$^{\Psi}$	NA	30
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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

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Colleagues,

We have learned with great pleasure and gratification that our journal, the Turkish Journal of Obstetrics and Gynecology, has been accepted to PubMed, one of the most respected and renowned medical indexes. I should sincerely emphasize that the tireless struggle and devoted endeavour of the journal editorial board and the editorial manager has an undisputed role in this success. Nevertheless, at least as precious as those efforts, is the role of the invaluable scientific contributions of obstetrics and gynecology physicians to our journal, which cannot go unseen in this achievement. In this context, on behalf of the Turkish Society of Obstetrics and Gynecology board, I congratulate the obstetricians and gynecologists, who contribute their scientific publications to our journal as well as the editorial board and the editorial manager of the Turkish Journal of Obstetrics and Gynecology, and thank them all for their efforts.

Even so, in order for our journal to achieve an even better position and to become a world-renowned and respected women's health journal and so that our country can play a greater role in the scientific environment, we still feel the need for the invaluable scientific support of our esteemed obstetrics and gynecology physicians. We believe that the high-quality papers that our colleagues will submit and the references they make to the articles published in our journal will carry it upwards in the list of women's health journals in the coming years.

I hope that our scientific progress will further accelerate and I would like to remind you about the 16th National Gynecology and Obstetrics Congress, which will be held in Antalya on 9-13 May 2018, in order for you to arrange your schedules accordingly, looking forward to being together once again for a great congress.

Sincerely,
Ateş Karateke, Prof. MD
President of TSOG

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

I am glad to announce that our journal "Turkish Journal of Obstetrics and Gynecology" is now indexed in "PubMed Central". It's been 13 years since the publication of the first issue and our journal is evolving and progressing. This is another milestone in the journey of a journal to become an international scientific venue. With the growing numbers of young scientists we will inevitably be in a highly ranked position among the relatively young journals.

Turkish Society of Obstetrics and Gynecology is launching a campaign to decrease preventable maternal deaths in order to reach the goal of less than 10 per 100.000 live births and to decrease the primary cesarean section rate by 10% a year. In order to help our colleagues to improve their capabilities to prevent obstetric hemorrhages, a fresh cadaveric course was held in İstanbul. A total of 150 specialists were trained on the management of obstetric hemorrhage, sepsis, disseminated intravascular coagulation, surgical anatomy, uterine devascularization surgeries to prevent bleeding, tamponades, catheter applications, uterine packing sutures on models and fresh cadavers. Organization of obstetric emergency teams is going on. In order to support this action we are calling for manuscripts conducted on the area of preventing maternal deaths and decreasing cesarean deliveries.

I hope this action will find supporters among you.

Best wishes,
Eray Çalışkan, Editor

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.92604

Turk J Obstet Gynecol 2017;14:145-50

Spectroscopic analysis of embryo culture media for predicting reproductive potential in patients undergoing *in vitro* fertilization

In vitro fertilizasyon hastalarının üreme potansiyelini belirlemede embriyo kültür medyasının spektroskopik analizle incelenmesi

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Abstract

Objective: To predict the reproductive potential of embryos via Raman spectroscopy evaluation of the spent culture media as well as with a conventional morphologic evaluation.

Materials and Methods: Women of reproductive age (n=31) who were treated for unexplained infertility and scheduled for single embryo transfer were invited to participate in this prospective study. After the embryos were removed from the culture, the spent culture media were stored at -80 °C after snap-freezing in liquid nitrogen.

Results: Fifteen patients were clinically pregnant, and 16 patients were clinically non-pregnant. Clinical pregnancy was predicted using Raman spectroscopy in 93% (14/15) of clinically pregnant patients, and in 62.5% (10 out of 16) of clinically non-pregnant patients. The sensitivity of the Raman spectroscopic analysis was 93% and the specificity was 62.5%.

Conclusion: Metabolomic evaluation of spent embryo culture media is an emerging technique with promising objective results. However, there is clearly room for improvement.

Keywords: *In vitro* fertilization, embryo culture media, morphology, Raman spectroscopy

Öz

Amaç: Embriyoların üreme potansiyelini belirlemede kültür medyasını Raman spektroskopik analizle ve süregelen morfolojik değerlendirmeyle karşılaştırılmasıdır.

Gereç ve Yöntemler: Açıklanamayan infertilite tedavisine göre ve tek embriyo transferi olacak 31 üreme yaşındaki kadın bu prospektif çalışmaya katıldı. Embriyolar kültürden alındıktan sonra, kültür medyası -80 °C'de likit nitrojende dondurulduu.

Bulgular: On beş hastada klinik gebelik bulunmuşken, 16 hastada gebelik bulunmamıştır. Klinik gebelik tahmini Raman spektroskopiyle %93 (14/15), gebe olmayan hastalarda ise %62,5 (10/16) olmuştur. Raman spektroskopik analizin sensitivitesi %93, spesifitesi %62,5 şeklindedir.

Sonuç: Embriyo kültür medyasının metabolomik değerlendirmesi objektif sonuçlar doğurabilir. Öte yandan metodun geliştirilmesi ihtiyacı gerekmektedir.

Anahtar Kelimeler: *In vitro* fertilizasyon, embriyo kültür medyası, morfoloji, Raman spektroskopi

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Received/Geliş Tarihi: 26.01.2017 **Accepted/Kabul Tarihi:** 06.09.2017

PRECIS: Metabolomic evaluation of spent embryo culture media is an emerging technique with promising objective results; however, there is clearly room for improvement.

Introduction

The identification of embryos with the highest potential to implant and establish ongoing pregnancy is the primary aim in human-assisted reproduction. This task is undertaken every day by embryologists worldwide during the treatment of couples that wish to conceive through in vitro fertilization (IVF). The optimal scenario is the transfer of a single embryo that gives rise to a singleton pregnancy. However, even in patients with good prognosis, namely patients aged under 35 years, implantation and the subsequent success rates are well below the expectations of infertile couples undergoing IVF treatment. Therefore, multiple embryo transfer has become clinical routine in IVF clinics worldwide to increase the rate of pregnancies. This approach has consequences such as an increased rate of multiple gestations, which are regarded as high-risk pregnancies[1]. Furthermore, the treatment process may cause considerable emotional, financial, and physical distress for couples undergoing IVF treatment because nearly two out of every three IVF cycles do not result in pregnancy[2]. The current limitations in the determination of embryos that have the highest implantation potential probably contribute to the low rates of pregnancy during IVF treatment. Hence, improvement of embryo selection has been a "hot research topic" since the beginning of IVF.

Morphology has been a very obvious parameter to assess embryos because it provides a chance for their evaluation from the oocyte stage, all the way to the blastocyst stage. Thus, in the first era of IVF, there was a number of studies that evaluated this parameter and associated morphology with IVF success rates. On the other hand, some researchers stated that the slight increase in pregnancy rates during IVF treatment was most likely a result of better practice in the laboratory than morphologic evaluation[3]. Due to the limitations of morphologic evaluation, several researchers investigated adjunctive non-invasive approaches for the assessment of the embryo, such as metabolic parameters[4].

Raman spectroscopy is a vibrational technique that has been used in the field of metabolomics, as well as other techniques such as near-infrared (NIR) spectroscopy, mass spectroscopy, and various others. Raman techniques use a laser to gather vibrational strokes or anti-strokes together with vibrations from the analyzed molecules. Laser-illuminated samples produce scattered light. The amount of light absorbed at a number of vibrational frequencies is then calculated. In the field of metabolomics, one advantage of the Raman technique is that evaluation of liquid samples is straightforward because water gives out weak signals.

The purpose of the present study was to predict the reproductive potential of embryos through Raman spectroscopy evaluation of the spent culture media, as well as conventional morphologic evaluation. We hypothesized that adding Raman spectroscopy to morphologic evaluation would predict better results than those of conventional morphologic evaluation alone.

Materials and Methods

Patients

Women of reproductive age (18 to 35 years) who were treated for unexplained infertility in the Infertility Clinic of İstanbul University Faculty of Medicine (İstanbul, Turkey) were invited to participate in this prospective study. Inclusion criteria for the diagnosis of unexplained infertility were as follows: (1) duration of infertility for >1 year; (2) confirmation of regular menstrual cycle, (2) hysterosalpingogram-confirmed presence of normal tubal patency; (3) normal day-3 hormonal panel of follicle-stimulating hormone and estradiol, and total antral follicle count more than 7 (4); normal semen analysis results according to the 2010 World Health Organization criteria[3]; (5) planned to undergo IVF/intracytoplasmic sperm injection (ICSI) treatment; and (6) planned to undergo day-3 embryo transfer. This study was approved by Ethics Committee of İstanbul University Faculty of Medicine (2015) and informed consent was received from all participants.

All patients received the same ovulation stimulation and monitoring protocols as previously described[5]. In brief, the patients underwent ovarian stimulation with a conventional gonadotropin-releasing hormone antagonist protocol.

After oocyte retrieval, cumulus complexes were isolated in the embryology laboratory and mechanically stripped. Afterwards, oocytes were put into separate 50 mL of culture (Sagew, Quinn's advantage protein plus cleavage medium, Cooper Surgical, Inc., Trumbull, CT, USA). The same culture was used in all embryos. Conventional ICSI was used in all patients according to our laboratory's process. After the presence of fertilization was identified on day-2 and day-3, as assessed by morphologic evaluation, developed embryos were put into separate 50 mL of culture until the cleavage stage. Throughout the study, standard tri-gas incubators that provided a 5% oxygen environment were used and all embryos were cultured in separate media.

Morphologic evaluation

Grade-1 embryos were transferred and analyzed according to the morphologic classification as established by Depa-Martynow et al.[6]. Grade-1 was considered as embryos with ≥7 blastomeres similar in shape and with <20% of cytoplasmic fragmentation.

End-point

Clinical pregnancy was the primary outcome of the study, which was defined as the presence of a fetal heartbeat using vaginal ultrasound at 6 weeks of amenorrhea.

Study design

After the embryos were removed from the culture and prepared for transfer, the spent culture media were loaded into separate labeled cryovials (Nunc Intermed, Kamstrup, Denmark), and stored at -80 °C after snap-freezing in liquid nitrogen. Cryovials were shipped on dry ice to the Raman Spectroscopy Laboratory at İstanbul Technical University (İstanbul, Turkey).

Raman spectroscopy

The thirty-one samples of 31 patients that were shipped to the Raman Spectroscopy Laboratory for measurement were kept at room temperature for a few minutes and then poured into custom-designed disk-shaped sample cells. The liquid samples were filled in a volumetric cylinder with a diameter of 1.6 mm and length of 6 mm. These cells allowed small volumes with a reservoir of 30 µL and consequently resulted in a long optical path for Raman scattering. The samples were exposed to a single longitudinal mode, 785 nm diode laser, whose output power was 100 mW. Inelastically-scattered photons were collected with a lens (scattering in 1800 geometry) and then focused into the 100-µm entrance slit of a spectrograph with another lens. The inserted photons were dispersed with a grating (600 l/mm) according to the wavelengths in the spectrograph and were imaged on a charge-coupled device (CCD) camera.

The spectra to be formed on the CCD camera, which were the multiple images of the entrance slit, were registered. Measurements were performed sequentially over 10 minutes. After a cleaning procedure, a distilled water spectrum was measured as background before every measurement in the same sample cell. A toluene spectrum was measured after every spent culture measurement in order to check laser stability and to have a reference for Raman calibration.

Spectral and statistical analyses

The Raman spectra were preprocessed using homemade software written on MATLAB and Simulink software (Mathworks, Natick, MA, USA). The spectral analysis preprocess steps are summarized in Figure 1. In brief, the spectra were first cleaned of unwanted cosmic ray peaks. After calibration of the wavenumbers, water spectra were subtracted from the embryo culture measurements as background correction. The residual fluorescence background profile of each spectrum was corrected using a third order polynomial and normalized to their maximum intensity.

Band component analysis was applied on the preprocessed spectra for 815-1065 cm^{-1} and 1140-1500 cm^{-1} regions using Gaussian line profiles. The bands were statistically analyzed in view of the pregnancy rates. Each band was tested using the Mann-Whitney U test. Only the band ratio of 900/940 cm^{-1} was statistically significant ($p<0.5$) (Figure 2).

Principal component analysis (PCA) together with quadratic discriminant analysis (QDA) were applied to the measurements for regions containing 900 and 940 cm^{-1} bands. The numbers of variables were picked as low as possible because PCA is affected by low sample numbers. The analysis was performed for a partial region of the spectra between 890 and 950 cm^{-1} where the most significant bands found in the band component analysis were located. The first two principal components were used as inputs for QDA analysis. A leave-one-out cross validation was applied to the QDA classifiers in order to obtain the best model. Receiver operating characteristic (ROC) analysis was performed on the QDA classifiers to test the accuracy of the analysis and to find a cut-off value between the two groups.

A sample size calculation was not conducted a priori because previous studies reported no significant differences between groups that were analyzed with conventional morphology and spectroscopic methods[7].

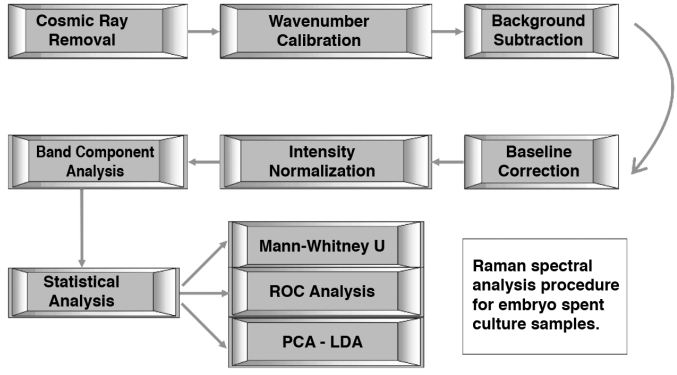

Figure 1. Raman spectral analysis procedure for embryo culture samples

ROC: Receiver operating characteristic, PCA: Principal component analysis, LDA: Linear discriminant analysis

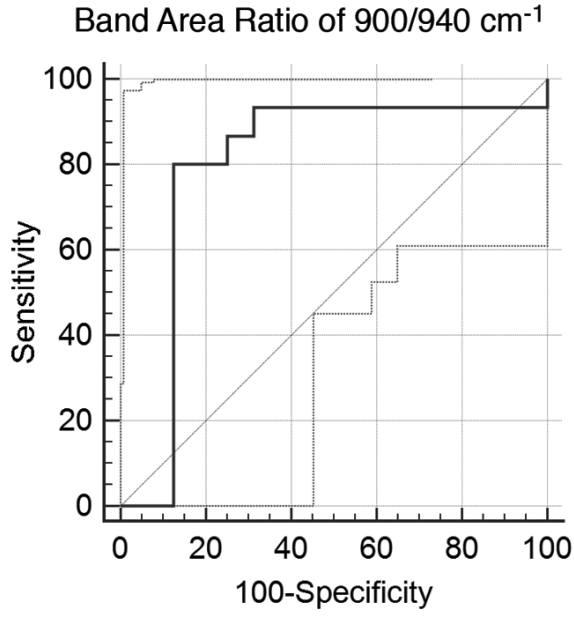

Figure 2. Receiver operating characteristic analysis was made for the band area of 900/940 cm^{-1}. The faint lines show the 95% confidence interval

Results

Thirty-one spent embryo culture media from 31 patients who met the inclusion criteria of the study were preprocessed.

The averages of Raman spectra were calculated for the pregnant and non-pregnant groups. The average spectra are demonstrated in Figure 3 for group comparison. The spectra show no clear distinction but was shifted for better visualization between the samples of patients whose embryos developed to pregnancy or not.

The similarity between the groups described above underscores the need for sophisticated statistical methods such as Raman spectral analysis. We applied QDA after PCA for the region between 890 and 950 cm^{-1}. The score plot of this analysis is shown in Figure 4. Each discriminant line is a parabola that maximizes the between-group distance and minimizes the within-group distance because QDA is a discriminant analysis method that uses second order polynomial function.

The accuracy of PCA-QDA analysis was tested with an additional ROC analysis. The curve obtained from the analysis is shown in Figure 5. ROC analysis was performed and a threshold of 0.4007 (band area ratio) was found. This curve shows that the optimal specificity and sensitivity of this accuracy ROC analysis was 80.25% and 87.50%, respectively.

Fifteen patients were clinically pregnant, and 16 patients were clinically non-pregnant (Table 1). Clinical pregnancy was predicted using Raman spectroscopy in 93% (14/15) of clinically pregnant patients, and in 62.5% (10 out of 16) of clinically non-pregnant patients. The sensitivity of the Raman spectroscopic analysis was 93% and the specificity was 62.5%.

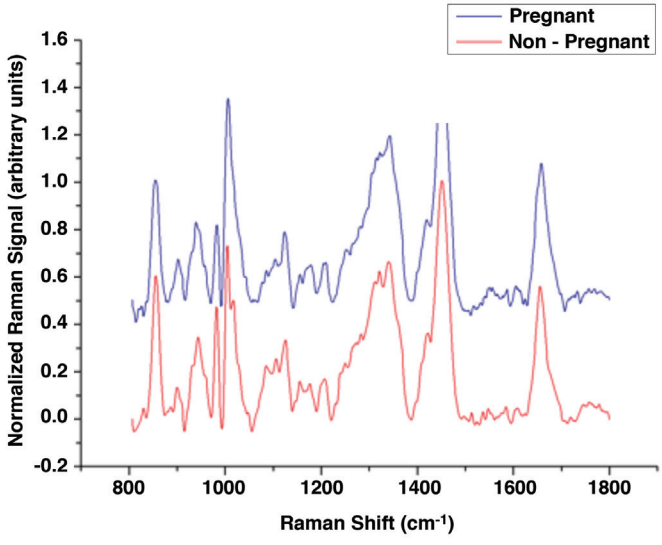

Figure 3. Raman spectra of two selected samples are demonstrated. The corresponding spectra of embryos that developed to pregnancy were manually shifted for better visualization

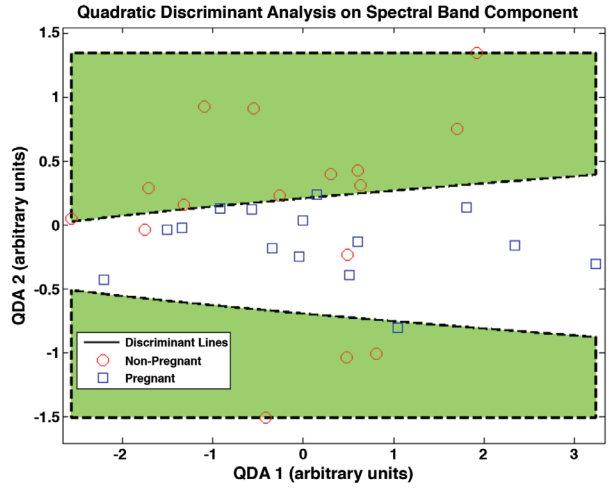

Figure 4. Quadratic discriminant analysis on the band area ratios obtained from band component analysis of the Raman spectra of 31 samples. Discriminant lines were calculated using home-made software that minimizes within-group distances and maximizes between-group distances

QDA: Quadratic discriminant analysis

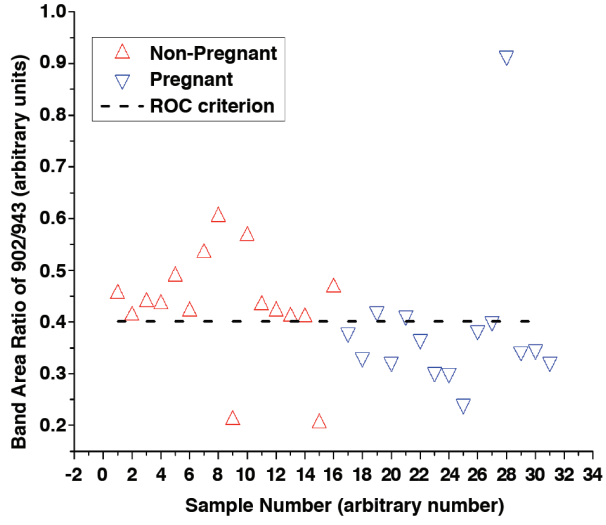

Figure 5. Receiver operating characteristic analysis was performed and a threshold of 0.4007 was found

ROC: Receiver operating characteristic

Table 1. Clinical pregnancy rates according to conventional morphologic evaluation and Raman spectra analysis

	Patients predicted to be pregnant according Raman spectra, n (%)	Patients predicted to be non-pregnant according Raman spectra, n (%)
Clinically pregnant patients, n (%)	14 (93)	1 (7)
Clinically non-pregnant patients, n (%)	6 (37.5)	10 (62.5)

Discussion

The present study identified that adding Raman spectroscopic analysis of spent embryo culture media revealed that this approach may predict clinical pregnancy as an adjunct to morphologic evaluation.

Currently, conventional morphologic evaluation is the most commonly used approach in assessing embryo quality worldwide. It has been used since the beginning of IVF, first as a tool to explain embryo development[8], and then to select the embryo with the highest implantation transfer potential[9-13]. Morphologic evaluation is based on the embryologist; therefore, embryo scores can vary considerably because it is still a somewhat subjective matter[14].

Metabolomics is an emerging "omics" science that has evolved from proteomics, genomics and transcriptomics. It systematically analyzes the inventory of metabolites. These metabolites are representatives of functional phenotypes at a cellular level. Within this context, researchers focused on the composition of the culture media. The subsequent step was the metabolomic analysis of what embryos use from the culture media and release during their development and to understand if there was a correlation with successful or unsuccessful implantation to the maternal uterus[15].

Seli et al.[16] documented the first proof-of-concept study, which correlated the metabolome of the spent embryo culture media with embryo viability using Raman spectroscopy. The mean spectrum of embryos that did not implant was compared with the mean spectrum of embryos that implanted successfully. In a study by Scott et al.[17], the mean spectrum was validated by analyzing spent embryo culture from a different IVF center that used a different type of culture. Further studies with either Raman and/or NIR spectroscopy argued that the metabolomic profile of the spent embryo culture media was a parameter that was independent of embryo morphology[18-24]. The present study is in line with these findings and shows that metabolomic evaluation of spent embryo culture media alone can predict reproductive potential as efficiently as conventional morphologic evaluation of the embryo.

Interestingly, despite the promise of metabolomic evaluation, the use of Raman spectroscopy and/or other techniques such as NIR and mass spectroscopy have limited clinical presence. There are a few potential explanations for these limitations. Currently, the equipment used for these spectroscopic techniques are expensive and require dedicated specialists. Moreover, prompt results are needed during embryo transfer in clinical practice and the applicable information gathered from spectroscopic techniques still requires time. If we look specifically at Raman spectroscopy for the purpose of metabolomic evaluation, it provides weak signal intensity, which may be a drawback when sample concentrations are low. Enhancement methods such as surface-enhanced Raman spectroscopy (SERS) or resonance Raman spectroscopy (RRS) can be used to overcome this limitation. On the other hand, RRS may damage the sample and produce broad background in the spectra; therefore, it may shield information from the spectra. Although SERS suffers from repeatability, it is a developing method and may be useful for future studies. However, it is important to note that even with the aforementioned drawbacks, Raman spectroscopy alone still produced results that were comparable to conventional morphologic evaluation in the present study. Hence, if these drawbacks were to be overcome in future studies, metabolomic evaluation could provide a more objective approach to the current morphologic evaluation and possibly result in becoming a more accurate technique with increased reproductive outcomes.

Study Limitations

A limitation of the study was its small sample size. We wanted to create an ideal design by only including single embryo transfer. Due to the financial aspects of IVF treatment and psychological burden, single embryo transfer is only occasionally feasible. Also, Raman spectroscopy equipment use and evaluation of embryo culture media required time to give results that could be utilized in routine clinic practice.

Conclusion

Existing embryo assessment relies heavily on the morphologic evaluation of the embryo by an embryologist. Furthermore, this subjective method does not provide sufficient specificity or sensitivity to produce desirable pregnant rates for patients receiving IVF treatment. Metabolomic evaluation of spent embryo culture media is an emerging technique with promising objective results. However, there is clearly room for improvement in the exact spectroscopic technique used for metabolomic evaluation. Moreover, it has to be adequately validated. Hence, this approach still remains experimental and its application has not translated into the clinical setting. Further randomized control trials with improved spectroscopic techniques are needed to document the potential benefit from the use of metabolomic evaluation alone or as an adjuvant approach to the conventional morphologic evaluation.

Acknowledgement

We would like to thank David F. Chapman, BSc, for editing the language of the manuscript. This research received funding from the Scientific Research Projects Coordination Unit of İstanbul University (grant number: 22501).

Ethics

Ethics Committee Approval: This study was approved by Ethics Committee of İstanbul University Faculty of Medicine (2015).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: E.B., G.B., Design: E.B., F.B., Data Collection or Processing: H.Y., S.B., Analysis or Interpretation: N.B., U.P., Literature Search: F.S., E.B., Writing: E.B., U.P.

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

Financial Disclosure: This research received funding from the Scientific Research Projects Coordination Unit of İstanbul University (grant number: 22501).

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.10734

Turk J Obstet Gynecol 2017;14:151-5

Micronuclei frequencies in lymphocytes and cervical cells of women with polycystic ovarian syndrome

Polikistik over sendromlu kadınlarda lenfositler ve servikal hücrelerde mikronükleus frekansları

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Abstract

Objective: The aim of this study was to determine micronucleus (MN) frequencies in exfoliated cervical cells and peripheral blood lymphocytes of women with polycystic ovarian syndrome (PCOS).

Materials and Methods: Fifteen patients with PCOS and 11 healthy control patients were included in the study. Cervical smears and peripheral blood were collected from all patients. Specimens were analyzed for MN frequencies and compared between the groups. In addition to MN, other nuclear anomalies connected with both genotoxicity and cytotoxicity were evaluated.

Results: The MN frequencies in cervical smear and peripheral blood lymphocytes were compared in patients with PCOS and normal controls. There was no statistically significant difference between the groups regarding micronucleus frequency in peripheral blood lymphocytes (p=0.239). The mean MN scores in exfoliated cervical cells of patients with PCOS and normal controls were 1.19±0.57 and 0.74±0.34, respectively. The difference regarding micronucleus frequencies in cervical cells was statistically significant between the groups (p=0.032).

Conclusion: Although study group is small, our study results support that there is an increased micronucleus frequency in cervical exfoliated cells of PCOS patients; this is a determinant of genetic hazard in the disease.

Keywords: Micronucleus tests, polycystic ovarian syndrome, cervical smears, lymphocytes, genotoxicity tests

Öz

Amaç: Bu çalışmanın amacı, polikistik over sendromlu (PKOS) kadınların eksfoliyatif servikal hücrelerinde ve periferik kan lenfositlerinde mikronükleus (MN) frekanslarını belirlemektir.

Gereç ve Yöntemler: PKOS'li 15 hasta ve 11 sağlıklı kontrol hastası çalışmaya dahil edildi. Tüm hastalardan servikal smearleri ve periferik kan toplandı. Numuneler, MN frekansları açısından analiz edildi ve gruplar arasında karşılaştırıldı. Hem MN sıklığı, hem de genotoksisite ve sitotoksite bağlı diğer nükleer anomaliler değerlendirildi.

Bulgular: Servikal smear ve periferik kan lenfositlerinde MN frekansları PKOS hastaları ve normal kontrollerde karşılaştırıldı. Periferik kan lenfositlerinde MN frekansı açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu (p=0,239). PKOS hastalarının eksfoliyatif servikal hücrelerindeki ortalama MN skorları ve normal kontrollerinki sırasıyla 1,19±0,57 ve 0,74±0,34 idi. Servikal hücrelerdeki MN frekansları istatistiksel olarak gruplar arasında anlamlıydı (p=0,032).

Sonuç: Çalışma grubu küçük olmasına rağmen çalışma sonuçlarımız, PKOS hastalarının eksfoliyatif hücrelerinde, hastalıkta genetik tehlikenin belirleyicisi olan MN sıklığının arttığını desteklemektedir.

Anahtar Kelimeler: Mikronükleus testleri, polikistik over sendromu, servikal yaymalar, lenfositler, genotoksisite testleri

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Received/Geliş Tarihi: 25.07.2017 **Accepted/Kabul Tarihi:** 16.09.2017

This study was presented at the 11th National Gynaecology and Obstetric Congress as a poster.

PRECIS: By using micronucleus (MN) genotoxicity tests, we determined MN frequencies in exfoliated cervical cells and peripheral blood lymphocytes of women with polycystic ovarian syndrome.

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy in women and it is characterized by chronic oligoanovulation and clinical/biochemical hyperandrogenism. Its prevalance is reported to be around 3.5-10% regarding diagnostic criteria[1].

Micronucleus (MN) is cytoplasmic chromatin condensations that appear as small nuclei, which are secondary to chromosomal fragmentation at the anaphase stage of cell division. MN is one of the established genotoxicity biomarkers in human erythrocytes, lymphocytes, reticulocytes, and exfoliated mucosa cells. Spontaneous or baseline MN frequencies in cultured human lymphocytes and exfoliated cells provide an index of accumulated genetic damage occurring during the life span of these cells. An increased frequency of MN is used as a measure to detect clastogenicity and aneugenicity. MN presence in cells reflects structural and/or numerical chromosomal aberrations arising during mitosis[2]. The genetic basis of PCOS has been investigated in several studies, and there is evidence of the presence of multiple gene polymorphisms, oxidative stress, and environmental factors in the background[3,4]. In patients with PCOS, increased oxidative stress and decreased antioxidant capacity have been reported, and those were all reported to be related to genetic damage in PCOS[5].

Yesilada et al.[6] reported that the MN frequency in peripheral blood lymphocytes of women with PCOS was increased. In our study, we aimed to investigate the micronuclei frequency both in peripheral lymphocytes and cervical squamous cells of patients with PCOS in order to demonstrate genetic damage, also in cervical smears.

Materials and Methods

The study was conducted Necmettin Erbakan University Meram Faculty of Medicine, Department of Gynecology, and Department of Medical Genetics between January 2012 and August 2012. Fourteen patients with PCOS and 11 controls with similar age and body mass index were recruited. PCOS was diagnosed using the Rotterdam criteria established in 2003. Patients with diseases related to hyperandrogenism such as hyperprolactinemia, Cushing's disease, androgen-secreting tumors, and late-onset congenital adrenal hyperplasia were all excluded by their medical history and specific tests. All participants answered a modified version of the questionnaire of the Commission for Protection against Environmental Mutagens and Carcinogens[7]. Information about contraceptive methods used, histories of sexually-transmitted diseases, and the patients' habits (smoking, drug use and numbers of sexual partners) were obtained. None of the patients included in the study had any systemic disease or history of smoking. Patients with multiple partners, abnormal cervical cytology results or history of genital warts were excluded from the study. All patients in the study had at least one negative smear test previously.

Informed consent was taken from all patients and the study was approved by Necmettin Erbakan University Meram Faculty of Medicine Research Ethics Committee (approval number: 2012/21).

Sampling and scoring of exfoliated cervical cells

Exfoliated cervical cells were collected using brushes and transferred to Falcon tubes containing 0.9% serum physiologic for MN tests. The material was centrifuged and the supernatant was discarded, leaving the exfoliated cells in the pellet. Cells were treated with hypotonic solution for 5 min. They were then treated twice with 5 mL of freshly prepared cold methanol: acetic acid (3:1). Drops of the material were placed on cold damp slides and allowed to dry. Samples were stained using 5% Giemsa for 5 minutes.

The slides were analyzed and 1000 epithelial cells were counted at a magnification of 1000 x (objective = 100 x with eyepiece = 10 x). Micronuclei were determined according to the criteria of Stich and Rosin[8]. Within the samples, only separate cells, without overlapping or folding, were analyzed. Micronuclei were counted if the structures had regular borders and were located inside the cytoplasm, with an intensity of staining less than or equal to that of the main nucleus and a size less than two-thirds of the size of the main nucleus (Figure 1a). The frequency of cells with micronucleus, binucleated cells (BNC), and cells with buds were reported as results.

Sampling and scoring of peripheric blood lymphocytes

Heparinized blood samples were obtained. Lymphocyte cultures were performed for each subject and incubated for 72 h at 37 °C. According to the cytokinesis-block human lymphocyte MN test, cytochalasin-B (Sigma) was added after 44 hours into the final concentration of 3 Gg/mL[9,10]. After a total of 72 h culture, cells were harvested. First, they were treated with prewarmed hypotonic solution (0.075 M KCl) for a few minutes at room temperature and then resuspended twice in cold fresh fixative (methanol:glacialacetic acid, 3:1). Fixed cells were dropped on clean slides. After air drying the slides, they were stained using 5% Giemsa for 5 min. For each sample, 1000 BNC were observed to assess the frequencies of MN (Figure 1b). The cells scored for MN had to be clearly seen as binucleate. The number of MN in each binucleate cell was scored[8]. MN were accepted only when (i) they were separated from the main nuclei, but included within the corresponding cytoplasm, (ii) they had a chromatin structure similar to that of the main nuclei, (iii) they were coplanar to the main nuclei[11], and (iv) they were no greater than one third the volume of the main nuclei[10,12].

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 18.0 (SPSS Inc., USA). Continous variables are expressed as mean ± standard deviation. Student's t-test was used to analyze statistical differences. The level of $p<0.05$ was considered statistically significant.

Results

The mean age in PCOS group was 29.3±5.2 years (range, 19-39 years), and in the control group it was 27.9±5.1 years (range, 21-35 years). The mean gravidity and parity in the PCOS group were 1.7±1.4 (range, 1-5) and 1.3±1.1 (range, 1-4) respectively, whereas they were 2.5±1.2 (range, 0-4) and 1.8±1.2 (range, 0-4) in the control group. There were no statistically significant differences between the groups regarding age, gravidity, and parity. The body mass indices were 25.1±3.4 kg/m^2 (range, 21-31 kg/m^2) in the PCOS group and 23.8±2.8 kg/m^2 (range, 21-28 kg/m^2) in the control group, respectively, without any statistical significance (p=0.147). The mean interval of coits was 4.7±3.6 in the PCOS group, whereas it was 5.1±3.1 in the control group; the difference was not statistically significant between the groups. The study results are summarized in Table 1. The MN frequency in the peripheral blood lymphocytes of women with PCOS was 10.93±6.5 (per 1000 cells), whereas it is 8.4±3.8 (per 1000 cells) in the control group. There was no statistically significant difference between the groups regarding MN frequency in peripheral blood lymphocytes (p=0.239). On the other hand, the MN frequency in cervical exfoliated cells was 1.19±0.57 (per 1000 cells) in PCOS group, whereas it is 0.74±0.34 (per 1000 cells) in the control group. The difference regarding MN frequency in cervical cells was statistically significant between the groups (p=0.032). In addition, other cellular discrepancies such as BNC and budding cells were 1.08±0.27 (per 1000 cells) and 0.10±0.08 (per 1000 cells) in the PCOS group, respectively, and 0.81±0.23 (per 1000 cells) and 0.06±0.06 (per 1000 cells) in the the control group. The difference between the groups regarding BNC was statistically significant (p=0.016), whereas the difference concerning budding cells was not statistically significant (p=0.147) (Table 2).

Discussion

PCOS, a common endocrinopathy among reproductive age women, is accepted to be associated with metabolic syndrome. It is also associated with severe long-term hazards such as type-2 diabetes, cardiovascular diseases, and endometrial

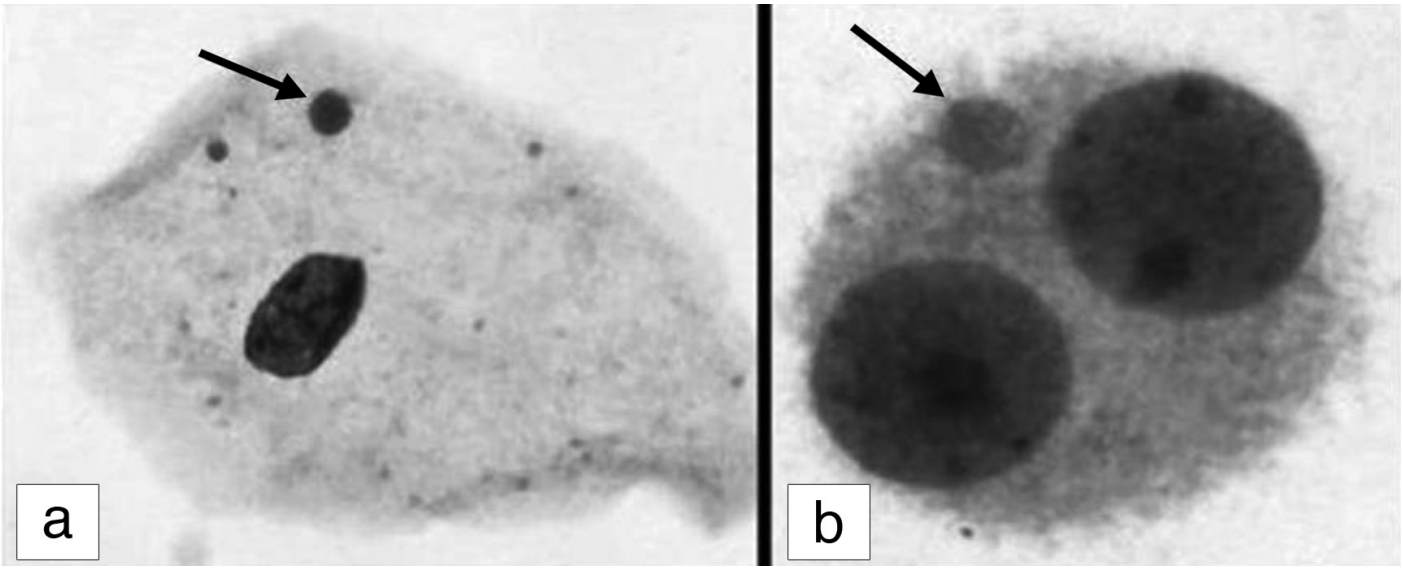

Figure 1. Micronucleus (arrow) in exfoliated cervical cell (a) and peripheral blood lymphocyte (b)

Table 1. Mean age, mean gravidity, mean parity and duration of sexual life in patients with polycystic ovarian syndrome and the control group

	PCOS group	Control group	p
Mean age (years) (mean + SD)	29.3±5.2 (19-39)	27.9±5.1 (21-35)	0.428
Mean gravidity (mean + SD)	1.7±1.4 (1-5)	2.5±1.2 (0-4)	0.148
Mean parity (mean + SD)	1.3±1.1 (1-4)	1.8±1.2 (0-4)	0.333
Duration of sexual lifea (mean + SD)	4.8±3.6 years	5.1±3.1 years	0.778

[a]Duration of sexual life in years from initial coitus; p<0.05
PCOS: Polycystic ovarian syndrome, SD: Standard deviation

Table 2. Average and standard deviation of the number of cells with micronuclei, binucleated cells, broken egg cells, and cells with buds

Anomalies	PCOS group	Normal controls	p
PBL MN frequency (mean ± SD)	10.93±6.5	8.4±3.8	0.239
ECC MN frequency (mean ± SD)	0.74±0.34	1.19±0.57	0.032*
BNC (mean ± SD)	1.08± 0.27	0.81±0.23	0.016*
BC (mean ± SD)	0.10±0.08	0.06±0.06	0.147

p<0.05; *there is a statistically significant difference
PCOS: Polycystic ovarian syndrome, PBL: Peripheric blood lymphocytes, MN: Micronucleus, SD: Standard deviation, ECC: Exfoliated cervical cells, BNC: Binucleated cells, BC: Cells with buds

cancer. Nowadays, PCOS and its relation with cancer is of special interest in scientific research. Oxidative stress due to an imbalance between the generation of reactive oxygen species and antioxidant defense has been investigated in the etiology of cancer development[13,14]. The imbalance between pro-oxidants and antioxidants has been proposed to be associated with reproductive diseases such as endometriosis, PCOS, and unexplained infertility. In the literature, there are various reports about increased oxidative stress and related comorbidity in patients with PCOS[5,15,16]. It is known that proteins, lipids, and DNA are exposed to damage as a result of oxidative stress. Moreover, in a study by Simic[14], it was defined that oxidative stress was linked to chromosomal breakage and carcinogenesis. Zuo et al.[17] reported that variable oxidative stress in PCOS, instability of genes, and DNA mutations increased the risk and potentially contributed to the pathogenesis of gynecologic cancers. Deepika et al.[18] reported that serum malondialdehyde levels of oxidative stress markers showed a positive correlation with MN in patients with PCOS.

There are also studies about the increased oxidative stress markers in patients with PCOS and this mechanism has been accepted to be related to mutagenesis in these patients[5,15]. Some investigators reported a positive correlation between oxidative stress and lipid peroxidation and genotoxicity[16-18]. This type of damage can lead to chromosomal losses and rearrangements. MN are DNA-containing particles that occur during mitosis and result from unrepaired DNA double-strand breaks, leading to chromatin fragments or whole chromosomes being distributed incorrectly. Therefore, the MN test shows genetic damage that cells accumulate throughout life. At the molecular level, this kind of DNA damage could be silenced tumor supressor genes and activated oncogenes. It could then initiate cancer development by adding epigenetic changes[17-19]. In our study, there was no statistically significant difference between the groups regarding MN frequency in peripheral blood lymphocytes (p=0.239). It is suggested that genetic abnormalities are present in women with PCOS. In previous studies, the micronuclei frequency in the peripheral lymphocytes of patients with PCOS was reported to be increased[6,20-23].

On the other hand, the MN frequency in cervical exfoliated cells was statistically significant between the groups (p=0.032). This study is the first to report on the genomic instability in cervical exfoliated cells of patients with PCOS. In the systematic review by Chittenden et al.[24], it was shown that women with PCOS were more likely to develop endometrial cancer. It has recently been reported that there may exist an association between PCOS and breast cancer and PCOS and ovarian cancer[25-27].

Cervical cancer is a significant health problem especially in developing countries. For prevention, early detection and treatment of preinvasive lesions that could progress to cervical cancer is very important. It has been shown exfoliated cervical cells of patients with moderate and severe dysplasia are observed with significantly higher frequency of MN compared with healthy women[28]. It may be suggested that the evaluation of the frequency of MN in exfoliated cervical cells may be helpful in establishing cervical cancer risk[29].

Study Limitations

Although the research has reached its aims, there were some unavoidable limitations. First, we had difficulty finding financial support for this research. For this reason, this study was conducted in a limited number of patients. Such studies should be carried out in wider series. Because of the same reason, we could not apply any additional test in assessing genotoxicity.

Conclusion

According to our study results, although the study sample was small, there is a genomic instability in cervical exfoliated cells of women with PCOS. This study stands as the the first in the literature concerning cervical cytology and genomic instability. Patients with PCOS should be followed up for cervical cancer with more frequent intervals than healthy women.

Ethics

Ethics Committee Approval: The study was approved by Necmettin Erbakan University Meram Faculty of Medicine Research Ethics Committee (approval number: 2012/21).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.K., K.G., F.K., Concept: R.K., A.G.Z., Design: R.K., A.G.Z., Data Collection or Processing: R.K., K.G., F.K., Analysis or Interpretation: A.G.Z., E.T., A.A., Literature Search: S.S., M.S.Y., Writing: S.S., M.S.Y.

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

Financial Disclosure: The authors declared that this study received no financial support. The remaining materails of previous studies were used for this study.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.54715

Turk J Obstet Gynecol 2017;14:156-9

No increase in free fetal DNA level in ectopic pregnancy: A preliminary study

Ektopik gebelik tanısında serbest fetal DNA'nın yeri: Ön hazırlık çalışması

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Abstract

Objective: The aim of this study was to diagnose ectopic pregnancy in the early period by measuring cell-free fetal DNA (cffDNA) levels in maternal blood using spectrophotometry.
Materials and Methods: Thirty patients with ectopic pregnancy and 30 patients with first trimester intrauterine pregnancy were enrolled in this prospective controlled study. cffDNA levels in maternal serum were measured using spectrophotometry.
Results: There were no differences between the two groups in terms of cffDNA absorbance levels.
Conclusion: Spectrophotometry is not suitable for measuring cffDNA levels to diagnose ectopic pregnancies in the early period. Practical and cost-effective methods should be found or larger patient series should be investigated.
Keywords: Ectopic pregnancy, free fetal DNA, spectrophotometry

Öz

Amaç: Ektopik gebelik tanısının erken dönemde spektrofotometrik yöntemle anne kanından serbest fetal DNA (cffDNA) ölçülerek konulmasıdır.
Gereç ve Yöntemler: Bu prospektif kontrollü çalışmada 30 adet ektopik gebelik tanısı almış hasta ile 30 adet ilk trimester intrauterin gebe cffDNA değerleri açısından spektrofotometrik yöntem kullanılarak karşılaştırıldı.
Bulgular: İki grup arasında cffDNA absorbans değerleri bakımından anlamlı fark bulunmadı.
Sonuç: Spektrofotometrik yöntemle cffDNA ölçümü ucuz ve basit bir yöntem olmasına rağmen ektopik gebeliğin erken tanısında yararlı bulunmamıştır. Polimeraz zincir reaksiyonu ya da daha hassas sitogenetik araştırmalar kullanılabilir; ancak maliyet etkinliği açısından uygun olmayabilir.
Anahtar Kelimeler: Ektopik gebelik, serbest fetal DNA, spektrofotometre

PRECIS: We have measured free fetal DNA level to diagnose ectopic pregnancy not to need repeat tests.

Introduction

Ectopic pregnancy is complication of pregnancy with high morbidity and mortality rates, which is why early and precise diagnosis is very important. The current administration for diagnosis of ectopic pregnancy includes serial serum beta human chorionic gonadotropin (β-hCG) levels and transvaginal ultrasound[1]. However, it may not be possible to distinguish between intrauterine or extrauterine pregnancy in 8-31% of cases at the first examination[2]. Thus, several checks for β-hCG level and ultrasound monitoring are required for diagnosis and management decisions. Another marker could be used in diagnosis and management. To date, many researchers have worked on this subject and reported a range of new markers that could be used in this issue.

In recent years, cell-free nucleic acids have been studied for use as potential candidate biomarkers for numerous conditions, especially in gynecologic cancers, ovarian and endometrial diseases, obstetric disorders such as preeclampsia[3], fetal aneuploidy, intrauterine fetal demise, and abortus[4]. Furthermore, concentration of cell-free nucleic acids in serum of patients undergoing *in vitro* fertilization

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Received/Geliş Tarihi: 28.05.2017 **Accepted/Kabul Tarihi:** 25.07.2017

(IVF) or embryo culture could give information about IVF outcomes[5].

In this preliminary study, we aimed to measure and compare cell-free fetal DNA (cffDNA) expression in the maternal circulation among women with intrauterine and ectopic pregnancies using spectrophotometry for cheap, easy, early, and precise diagnosis with only one blood test.

Materials and Methods

This study was performed with 30 patients with ectopic pregnancy and 30 women with first trimester singular pregnancies without any medical problems serving as controls who were admitted to the Gaziantep University Faculty of Medicine, Department of Obstetric and Gynaecology. The ectopic pregnancy group was named as group 1 and the control group was named as group 2. An acceptance form was signed all volunteer pregnant women. This study was approved by the Gaziantep University Local Ethics Committee (approval number: 05/2011-16).

Diagnostic criteria of ectopic pregnancy were; irregular β-hCG increase, ectopic implantation of pregnancy or diffuse fluid in the pouch of Douglas and abdomen, as determined using transvaginal ultrasound.

We took 8-10 cc blood samples from the intrauterine pregnancy group and ectopic pregnancy group, which were then placed in two 15 cc Falcon tubes and sent to the laboratory within two hours and centrifuged at 2480 rpm for 10 minutes. The supernatant with its pellet was then separated to another Falcon tube and centrifuged at 3600 rpm for 20 minutes. Finally, the supernatant was separated into 3 Eppendorf tubes (1.5 cc) with its pellet and kept at -80 °C until required for analysis. These materials were thawed for analysis and DNA absorption measurements were made using a Tetra Spectrophotometer, Model: T80+UV/VIS Spectrophotometer.

Statistical Analysis

Data were analyzed using SPSS 13.0. The independent t-test, independent Levene's test, and Mann-Whitney U test were used when appropriate. Statistical significance was considered as $p<0.05$.

Results

The average age of both groups was 24.9±4.8 years (range,16-36 years). The mean age of the ectopic pregnancy group was 25.3±4.5 years (range, 16-35 years). The mean age of the intrauterine pregnancy group was 24.4±5.1 years (range, 18-36 years). There was no statistically significant difference in terms of age between the two groups (p=0.776). The average weight of the both groups was 67.7±11.5 kg (range, 45-92 kg). There was no statistically significant difference in terms of weight (p=0.968), height (p=0.507), body mass index (p=0.873) and hemoglobin levels (p=0.741) between the two groups. The sociodemographic data of the groups are listed in Table 1 and Table 2. β-hCG levels were higher than in the control group

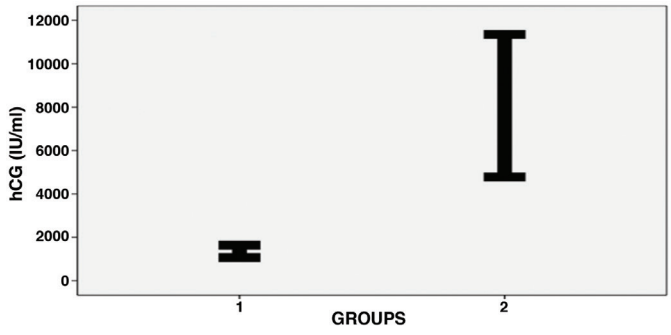

Figure 1. Beta human chorionic gonadotropin levels of two groups
hCG: Human chorionic gonadotropin

Table 1. Sociodemographic data of ectopic pregnancy group (group 1)

Variables	n/Total* (%)
Mother's education status	
Illiterate	2/30 (6.7%)
Literate	4/30 (13.3%)
Primary school graduate	4/30 (13.3%)
Secondary school graduate	9/30 (30%)
High school graduate	8/30 (26.7%)
University graduate	3/30 (10%)
Husband's education status	
Illiterate	0/30 (0%)
Literate	3/30 (10%)
Primary school graduate	5/30 (16.7%)
Secondary school graduate	9/30 (30%)
High school graduate	10/30 (33.3%)
University graduate	3/30 (10%)
Mother's job	
Housewife	21/30 (70%)
Working	9/30 (30%)
Husband's job	
Self-employment	18/30 (60%)
Employee	8/30 (26.7%)
Officer	4/30 (13.3%)
Social security	
No	4/30 (13.3%)
Yes	26/30 (8.7%)
Mother's chronic disease	
No	28/30 (93.3%)
Yes	2/30 (6.7%)

*: Abnormal values in both groups

and this was statistically significant (p<0.001) (Figure 1). There were no differences between the two groups with respect to DNA absorbance levels (p=0.647) (Figure 2).

Discussion

It is crucial to diagnose ectopic pregnancies in the earliest possible time because this interval of suspicion but uncertainity can cause dangerous conditions such as internal bleeding and future infertility[6]. Less than 50% of tubal ectopic pregnancies could be diagnosed at the first examination of patients[7,8]. We performed this research in the hope of identifying a new marker that could identify this important illness as early as possible. Therefore, we measured cffDNA absorbance using spectrophotometry, but we could found differenced between intrauterine the pregnancy and ectopic pregnancy groups in terms of cffDNA absorbance. We also found that the cffDNA values did not increase with increasing hCG values. This is a valuable result because it showed that the amount of the cffDNA could not give information about the week or settlement of the pregnancy. A previous study also reported that cffDNA was not suitable as a marker for pregnancy complications, but it was suitable for the diagnosis of aneuploidies[9].

Researchers are looking for many new markers to shorten the diagnosis time of ectopic pregnancy and reduce the possibility of tubal rupture. Previous studies researched placental (pregnancy-associated plasma protein A, human placental lactogen, inhibin A, activin A, vascular endothelial growth factor) and non-placental markers (glycodelin and vasculer endotelial growing factor) for the diagnosis of ectopic pregnancy[10,11]. Our study is the second regarding cffDNA in ectopic pregnancy. There is only one more study about cffDNA measurements in ectopic pregnancies. In that study, Lazar et al.[12] investigated free DNA quantities using polymerase chain reaction (PCR) analysis of sex-determining region Y (SRY) between ectopic and intrauterine pregnancies. SRY was found in 15 ectopic pregnancies and 14 intrauterine pregnancies. Mean fetal free DNA quantity in ectopic pregnancies has been found higher than in intrauterine pregnancies (p<0.005). This result is significant but PCR is an expensive and long-term method; therefore, it is not suitable for ectopic pregnancy.

Measurement of the amount of nucleic acids is an essential tool in molecular biology that uses quantities of DNA solutions ranging from 1 pg/µL to 50 mg/mL[13]. A previous study measures the DNA quantity of Enterobacter from intestinal

Table 2. Sociodemographic data of intrauterine pregnancy group (group 2)

Variables	n/Total* (%)
Mother's education status	
Illiterate	1/30 (3.3%)
Literate	1/30 (3.3%)
Primary school graduate	5/30 (16.7%)
Secondary school graduate	8/30 (26.7%)
High school graduate	8/30 (26.7%)
University graduate	6/30 (20%)
Husband's education status	
Illiterate	0/30 (0%)
Literate	1/30 (3.3%)
Primary school graduate	8/30 (26.7%)
Secondary school graduate	10/30 (33.3%)
High school graduate	10/30 (33.3%)
University graduate	1/30 (3.3%)
Mother's job	
Housewife	19/30 (63.3%)
Working	7/30 (23.3%)
Husband's job	
Self-employment	21/30 (70%)
Employee	8/30 (26.7%)
Officer	1/30 (3.3%)
Social security	
No	1/30 (3.3%)
Yes	29/30 (96.7%)
Mother's chronic disease	
No	26/30 (86.7%)
Yes	4 /30 (13.3%)

*: Abnormal values in both groups

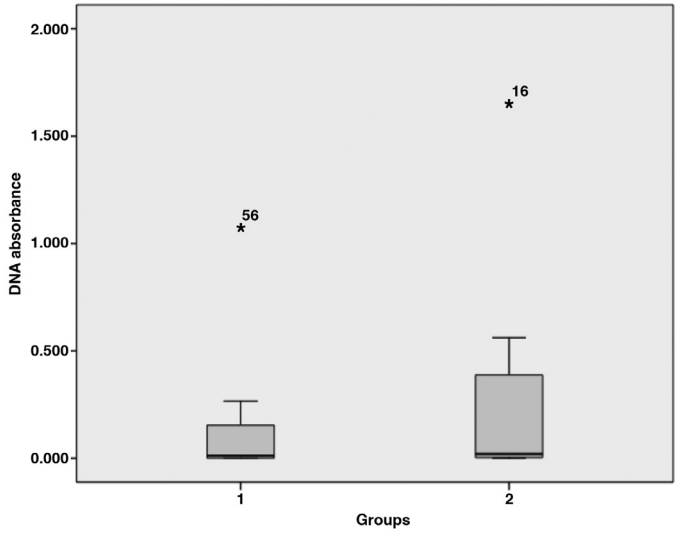

Figure 2. DNA absorbance levels of two groups

**Values that are abnormally high but not statistically significant between the two groups*

flora at 260 nm, but they used PCR at the beginning to extract genomic DNA[14]. We tried to measure cffDNA from maternal blood using spectrophotometry only. The reason that we found no meaningful result could be due to the fact that cffDNA in the blood of patients with ectopic pregnancy does not reach meaningful levels, different from intrauterine pregnancies, or that spectrophotometry only is insufficient without extracting cffDNA. Nevertheless, we foresee diagnosing ectopic pregnancy in the earliest period by measuring cffDNA with an easy, fast, and inexpensive method so we can use it in daily routine.

Study Limitations

Spectrophotometry to measure cffDNA is a cheap method, but it may not be intensive enough or we need larger patient groups for searching.

Conclusion

In conclusion, this pilot study may lead to other studies about cffDNA measurement for ectopic pregnancy in larger patient groups using spectrophotometry or any other easy, fast and cheap method.

The measurement of concentrations of cffDNA seems to be a promising tool for early diagnosis of ectopic pregnancy, but evaluation of the technique would be necessary. Spectrophotometry method is not suitable for measuring cffDNA levels to diagnose ectopic pregnancies in the early period. Practical and cost-effective methods should be found or larger patient series should be investigated for using cffDNA in the early diagnosis of ectopic pregnancy.

Ethics

Ethics Committee Approval: This study was approved by the Gaziantep University Local Ethics Committee (approval number: 05/2011-16).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.K.K., M.G.U., Concept: Ö.K.K., M.G.U., Design: Ö.B., Data Collection or Processing: Ö.K.K., Analysis or Interpretation: M.G.U., Literature Search: S.S., Writing: Ö.K.K.

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

Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.67674

Turk J Obstet Gynecol 2017;14:160-5

Can we predict severity of intrahepatic cholestasis of pregnancy using inflammatory markers?

Enflamatuvar belirteçleri kullanarak gebelik kolestazının ciddiyeti öngörülebilir mi?

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Abstract

Objective: To investigate the association of inflammatory markers with severity of intrahepatic cholestasis of pregnancy (ICP).
Materials and Methods: This retrospective case-control study was conducted with 229 pregnant women, 84 with ICP, and 145 age-matched healthy pregnant women. Patients were categorized as mild ICP (<40 µmol/L) and severe ICP (≥40 µmol/L) with regard to serum bile acids. Inflammatory markers (neutrophil-to-lymphocyte ratio (NLR), platelet-to- lymphocyte ratio (PLR) and mean platelet volume (MPV), and red blood cell distribution width (RDW) were compared between the groups.
Results: Patients with ICP had significantly decreased RDW and increased white blood cell counts (WBC), MPV and PLR, but no significant changes in NLR. The comparison of mild and severe cases with regard to NLR, PLR, WBC, and RDW was similar (p>0.05). MPV levels were significantly increased in severe group (p<0.05).
Conclusion: WBC, MPV, and PLR were the inflammatory markers significantly increased, and RDW was signifantly reduced in ICP. MPV was the marker that significantly increased with the severity of disease. The use of inflammatory markers in the assessment of perinatal outcomes needs further studies.
Keywords: Cholestasis, inflammation, mean platelet volume, platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio

Öz

Amaç: Gebeliğin intrahepatik kolestazının (GİK) ciddiyeti ile enflamatuvar belirteçler arasındaki ilişkiyi incelemektir.
Gereç ve Yöntemler: Bu retrospektif olgu-kontrol çalışması 84 GİK'li ve 145 yaş uyumlu sağlıklı gebe olan 229 gebe kadın üzerinde gerçekleştirilmiştir. Hastalar serum safra asit düzeylerine göre, hafif GİK (<40 µmol/L) ve ciddi GİK (≥40 µmol/L) olarak kategorize edilmiştir. Çalışmamızda bu iki grup arasında enflamatuvar belirteçler olan nötrofil-lenfosit oranı (NLO), platelet-lenfosit oranı (PLO), ortalama platelet hacmi (MPV) ve kırmızı kan hücreleri dağılım genişliği (RDW) karşılaştırılacaktır.
Bulgular: GİK hastalarında RDW azalmış, beyaz kan hücresi (WBC), MPV, ve PLO artmıştır, ancak NLO'da önemli bir değişiklik izlenmemiştir. Hafif ve ciddi GİK grupları arasında karşılaştırma sonucunda NLO, PLO, WBC ve RDW benzer bulunmakla beraber MPV seviyesi ciddi grupta anlamlı olarak yüksek tespit edilmiştir (p<0,05).
Sonuç: GİK hastalarında WBC, MPV, PLO anlamlı olarak artan, RDW ise anlamlı olarak azalan enflamatuvar belirteçlerdir. MPV ise hastalığın ciddiyeti ile birlikte artış gösteren bir belirteçtir. GİK hastalarında bu enflamatuvar belirteçlerin perinatal sonuçlarla ilişkisini incelemek için ise daha fazla çalışmaya ihtiyaç vardır.
Anahtar Kelimeler: Kolestaz, enflamasyon, ortalama platelet hacmi, platelet-lenfosit oranı, nötrofil-lenfosit oranı

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Received/Geliş Tarihi: 27.05.2017 **Accepted/Kabul Tarihi:** 16.09.2017

PRECIS: This paper investigated the hematological inflammatory markers in mild and severe cases of intrahepatic cholestasis of pregnancy. Mean platelet volume was significantly increased in severe intrahepatic cholestasis of pregnancy.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common liver disease seen during pregnancy, with a changing prevalence worldwide[1,2]. The etiology and pathogenesis of ICP are multifactorial. Environmental factors, nutritional deficiencies, hormonal changes, and genetic variations have been found to be responsible for ICP[3,4]. It presents most often in the form of pruritus in the second and third trimesters of pregnancy, with elevated serum aminotransferases and/or elevated serum bile acid levels (≥10 µmol/L)[5]. ICP can be differentiated from other types of liver diseases unique to pregnancy that share similar laboratory abnormalities such as preeclampsia, acute fatty liver of pregnancy, and hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome[1,6,7]. In addition, other skin diseases that cause high transaminase levels in pregnancy must be excluded. After delivery, the symptoms of ICP usually resolve within 48 hours, with laboratory abnormalities normalizing within 2-8 weeks[8,9].

Bile acid levels can affect perinatal outcomes and are related to an increased risk of iatrogenic preterm delivery, spontaneous preterm delivery, meconium-stained amniotic fluid (MSA), and sudden intrauterine death of the fetus[8,10-12]. In the severe ICP group, the incidence of complications is higher than in the mild group[8]. Unfortunately, ultrasonography, cardiotocography, fetal movements, and Doppler ultrasonography cannot predict fetal death; there is no perfect test or prognostic marker available to predict fetal outcome[8,13,14].

Recent studies demonstrated the prognostic role of inflammatory markers in both cardiovascular diseases and malignancies[15,16], but few studies have been performed with ICP. The neutrophil-to-lymphocyte (NLR) ratio, platelet-to-lymphocyte (PLR) ratio, mean platelet volume (MPV), and red blood cell distribution width (RDW) are hematologic inflammatory markers. One important pathogenesis responsible for the occurrence of ICP is inflammation; however, it is not known which mechanism initiates this inflammation[17]. Recently, NLR has been found to be a promising diagnostic marker in ICP[18]. To the best of our knowledge, no studies have investigated the roles of PLR and RDW in ICP. Therefore, we aimed to evaluate the role of inflammatory markers, which are readily available and easily calculated parameters, in the severity of ICP.

Materials and Methods

This retrospective case-control study was conducted at Zeynep Kamil Women and Children's Health Training and Research Hospital, İstanbul. Patients with ICP who delivered their babies in this hospital from January 2013 to January 2016 were enrolled in this study. All data were obtained from hospital files and our computer database. This study was approved by the Zeynep Kamil Women and Children's Health Training and Research Hospital Local Ethics Committee (approval number: 136).

The term ICP was used if the serum bile acid level was ≥10 µmol/L with pruritus that could not be explained by any other condition. A total of 102 women with ICP were enrolled in this study. The exclusion criteria were: patients with incomplete data, fetal congenital anomalies, multiple pregnancies, chronic/acute liver disease (Wilson's disease, cholecystitis, primary sclerosing cholangitis, primary biliary cirrhosis, alpha-1-antitrypsin deficiency, symptomatic cholelithiasis, cytomegalovirus, Epstein-Barr virus, autoimmune hepatitis, or acute fatty liver of pregnancy), and HELLP syndrome. A total of 84 singleton pregnancies were included in this research.

The patients with ICP were categorized into two groups according to their serum bile acid levels: mild (<40 µmol/L, n=53) and severe (≥40 µmol/L, n=31). The control group was selected from age-matched healthy women who had singleton deliveries on the same day as that of patient's with ICP. All gestational age-matched controls complied with the exclusion criteria (n=145).

Serum bile acids were evaluated using an enzymatic assay with intra and inter-assay precisions of 3% and 4%, respectively, [Diazyme Total Bile Acids (TBA) kit; Diazyme Diagnostic Laboratories, USA] and a Cobas C501 (Roche, USA). A blood analyzer (Cell-Dyn 3700; Abbott, USA) was used to determine the complete blood cell count (CBC). The CBC inflammatory markers measured were white blood cell counts (WBC), platelets, NLR, PLR, MPV, and RDW.

Perinatal death was defined as mortality from over 24 weeks' gestation until 7 days postpartum. A low Apgar score was defined as a score of below 7 at 5 minutes. The main outcome of the measures was the association of the inflammatory factors (WBC, NLR, PLR, MPV, and PDW) with the severity of ICP.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences for Windows version 18 (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was accepted as being statistically significant, and all measurements were performed within a 95% confidence interval. The results of the study are expressed as means, standard deviations, and percentages. According to the data distribution, comparisons were made using Student's t-test, ANOVA, or the chi-square (χ^2) test, when appropriate. A post-hoc least significant difference test was used after the ANOVA analysis. Relationships between the data were evaluated using Pearson's correlations.

Results

The comparison between the patients with ICP (n=84) and controls (n=145) showed similar ages (28±5.5 vs. 28.1±5.2

years, p>0.05) and gravidities (2.1±1.3 vs. 2.3±1.2, p>0.05). The ICP group showed significantly decreased gestational weeks at delivery (36.2±2.3 vs. 39.1±1.4 weeks, p<0.001) and birth weights (2899±623.3 g vs. 3373±413.9 g, p<0.001) when compared with the healthy controls. The comparison of the characteristic findings of the patients with mild and severe ICP is presented in Table 1. The majority of patients (77.3% mild ICP vs. 74.1% severe ICP) were overweight/obese and aged younger than 35 years (79.2% mild ICP vs. 70.9% severe ICP), respectively. The women with mild and severe ICP exhibited similar characteristics with regard to educational status, chronic disease history, and previous ICP history (p<0.05). Table 2 presents the laboratory findings; with the exception of serum bile acid levels, the women with mild and severe ICP had similar findings.

Table 1. The basal characteristics of mild (n=53) and severe (n=31) intrahepatic cholestasis of pregnancy

	Mild ICP	Severe ICP
Age <35 years	42 (79.2%)	22 (70.9%)
BMI (overweight/obese) (kg/m^2)	41 (77.3%)	23 (74.1%)
Nulliparity	33 (62.5%)	11 (35.4%)
Abortion	17 (32%)	8 (25.8%)
Education (primary school)	38 (71.6%)	26 (83.8%)
Prior ICP	4 (7.5%)	2 (6.4%)
IVF pregnancy	3 (5.8%)	2 (6.4%)
Progesterone use in pregnancy	13 (22.6%)	5 (16.1%)

ICP: Intrahepatic cholestasis of pregnancy, BMI: Body mass index, IVF: *In vitro* fertilization

Table 2. The comparison of the laboratory findings between patients with mild and severe intrahepatic cholestasis of pregnancy

	Mild ICP (n=53)	Severe ICP (n=31)	p†
Hemoglobin (g/dL)	11.1±1.1	11.0±1.1	0.757
Hematocrit (%)	34.0±4.1	34.1±3.0	0.895
Alkaline phosphatase (ng/mL)	259±391.2	249±102.0	0.899
Lactate dehydrogenase (U/L)	24.3±71.0	25.0±51.0	0.648
Gamaglutamyl transferase (U/L)	27.7±28.7	23.8±14.5	0.518
AST (U/L)	135.1±139.2	144.8±173.1	0.773
ALT (U/L)	134.1±133.5	94.3±96.4	0.133
Bile acids (µmol/L)	20.5±7.6	78.9±50.0	<0.001
Fibrinogen (mg/dL)	54±105.0	52.0±128.0	0.743

†P value is obtained by t-test (Levene test used for homogeneity of variances)
ICP: Intrahepatic cholestasis of pregnancy, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Serum bile acids were positively and significantly correlated with PLR (r=0.343, p=0.003), but the correlations of bile acids with WBC (r=-0.062), neutrophils (r=-0.198), lymphocytes (r=-0.112), MPV (r=0.08), RDW (r=-0.174), and NLR (r=-0.110) were statistically non-significant (p>0.05). Serum bile acids were negatively significantly correlated with gestational age at delivery (r=-0.390, p<0.001) and birth weight (r=-0.252, p=0.02), and PLR was negatively correlated with gestational age at delivery (r=-0.254, p=0.003).

The comparison of the obstetric outcomes in the women with mild and severe ICP is given in Table 3. The percentages of those having male fetuses, low Apgar scores, fetal distress, MSA, preeclampsia, perinatal/neonatal mortality, gestational diabetes, Rh isoimmunization, and abortus imminence were similar between the women with mild and severe ICP. However, the gestational age at delivery and time of diagnosis were earlier in the severe group (p<0.05). In addition, the cesarean section rate was significantly increased in the severe group (p<0.05).

Table 4 shows the comparison of the inflammatory markers between the normal and ICP groups. Overall, the inflammatory markers were significantly increased in the ICP group, including WBC, MPV, and PLR (p<0.05), and neutrophils, lymphocytes, and RDW were significantly decreased in the ICP group (p<0.05). However, NLRs were similar between the normal and ICP groups.

Table 3. The comparison of obstetric outcomes in mild (n=53) and severe (n=31) intrahepatic cholestasis of pregnancy

Mean ± SD	Mild ICP	Severe ICP
Gestational age at diagnosis	(33.5±2.8)	(30.8±4.7)†
Gestational age at delivery	(36.7±1.2)	(35.3±3.2)†
Birth weight (g)	(2975±568)	(2733±653)
Cesarean rate n (%)	29 (54.7%)	26 (83.6%)†
n (%) Male fetus	20 (37.7%)	11 (35.4%)
Fetal distress	2 (3.7%)	4 (12.9%)
Low Apgar score	1 (1.9%)	2 (6.4%)
Meconium stained aminiotic fluid	2 (3.8%)	3 (9.6%)
Perinatal mortality	0 (0%)	1 (3.2%)
Neonatal mortality	1 (1.9%)	1 (3.2%)
Neonatal intensive care unit	10 (19%)	9 (29%)
Preeclampsia	7 (13.2%)	2 (6.4%)
Gestational diabetes	13 (24.5%)	4 (12.9%)
Rh isoimmunization	8 (15%)	4 (12.9%)
Hyperemesis gravidarum	1 (1.9%)	1 (3.2%)
Abortus imminence	7 (13.2%)	4 (12.9%)

P values were obtained using chi-square test or the t-test as appropriate (†significant at p<0.05 level)
ICP: Intrahepatic cholestasis of pregnancy, SD: Standard deviation

Table 5 presents the comparison of the inflammatory markers in the healthy controls and women with mild and severe ICP. The RDW (p=0.128) and WBC (p=0.535) values were similar between the women with mild and severe ICP. MPV was significantly increased in the severe ICP group when compared with controls and the mild ICP group (p<0.05). Despite the fact that PLR was increased and RDW was decreased in patients with ICP, they were not significantly changed between the mild and severe groups. MPV was the marker that significantly increased in the severe group.

Discussion

ICP is a liver disease of pregnancy that increases fetal mortality; therefore, early diagnosis and assessment of the severity of the disease is an important task. In this study, we aimed to investigate the associations between the readily available, but newly defined, inflammatory markers, NLR, PLR, MPV, and RDW, with the severity of ICP. The present study found that the inflammatory markers were significantly increased in patients with ICP, and that MPV increased with the severity of ICP.

Fetal distress, premature delivery, perinatal asphyxia, and intrauterine fetal death may all occur in patients with ICP[2,8,19], and increased bile acid levels are thought to be the cause of these complications[20]. Consistent with previous studies, we observed lower birth weights and more preterm deliveries among patients with ICP[11,21-23]. However, there were no significant differences found in maternal age, parity, diabetes history, history of chronic systemic disease, preeclampsia or maternal hepatitis B and C infections in the women with mild and severe ICP, which was consistent with the study by Kawakita et al.[24]. Some studies found similar cesarean section rates[12,25,26], in contrast to others[20,27]. In our study, cesarean rates were higher with severe ICP. Some authors found an increased MSA risk in the ICP group when compared with controls[8,28,29], but some authors did not support these findings[27]. In addition, some previous studies reported an increased MSA risk in the severe ICP group when compared with the mild group[26,27], but conflicting results have been reported[29] in the literature. Also in our study, we didn't find an increased MSA risk in the severe group when compared with the mild group. Fetal asphyxia in the newborns of patients with ICP has been reported frequently in the literature[30,31]. Overall, the characteristics of these patients show wide variations, and the findings are inconsistent in the literature[30].

Previous studies about ICP and inflammation suggested that ICP was an inflammatory process, and that perinatal outcomes were related to inflammation[18,31-33]. Bile acids are thought to be related to inflammation, and they directly affect hepatocytes and stimulate the secretion of proinflammatory mediators, which causes neutrophil accumulation, extravasation, and activation[17]. Prior studies used the hematologic markers WBC[18], MPV[31,32], and NLR[18] as inflammatory markers in ICP. However, as far as we know, no studies have investigated the relationships between ICP and PLR and RDW. Therefore, to the best of our knowledge, this paper is the first to determine the associations between ICP and all CBC inflammatory markers. The results showed that serum bile acids were positively and significantly correlated with PLR (r=0.343, p=0.003), and the ICP group had a significantly increased PLR and significantly

Table 4. The comparison of hematologic indices between control and intrahepatic cholestasis of pregnancy group

Hematologic parameters	ICP (n=84)	Control (n=145)	p†
Hemoglobin (g/dL)	11.0±1.1	10.0±1	0.11
Hematocrit (%)	34.1±3.7	34.3±3	0.66
Platelet (10^3/mm^3)	249±80	246±69	0.81
White blood cell count (10^3/mm^3)	17449±3932	10333±2259	<0.001
Neutrophil (10^3/mm^3)	7.2±2.2	7.9±1.9	0.04
Lymphocyte (10^3/mm^3)	1.8±0.5	2.0±0.5	0.01
MPV (f/L)	9.2±2.6	8.2±1.2	0.004
RDW (%)	15.1±2.7	17.1±3.4	<0.001
NLR	4.1±1.7	4.0±1.4	0.77
PLR	145±64	125±43	0.02

†P value is obtained by t-test (Levene test used for homogeneity of variances)
ICP: Intrahepatic cholestasis of pregnancy, MPV: Mean platelet volume, RDW: Red blood cell distribution width, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

Table 5. The comparison of inflammatory markers

	Control (n=145)	Mild ICP (n=53)	Severe ICP (n=31)	p†
WBC (10^3/mm^3)	10.360±2300	15.000±3670	21.4±4419	<0.001
Platelet (10^3/mm^3)	246±68.0	242±72.3	261±93.6	0.525
MPV (f/L)	8.2±1.2	8.7±1.9	10.1±3.4	>0.001
RDW (%)	17.1±3.4	15.5±2.6	14.3±2.8	>0.001
NLR	4.0±1.4	4.3±1.7	3.8±1.5	0.312
PLR	125.4±43.0	144.0±61.0	147.0±69.0	0.06

†P value is obtained by ANOVA test (post-hoc least significant difference is used for subgroup analysis)
WBC: White blood cell count, MPV: Mean platelet volume, RDW: Red blood cell distribution width, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

decreased RDW ratio when compared with the controls. Despite the increased PLRs and decreased RDWs in the patients with ICP, they were not significantly changed in severe ICP.

Kirbas et al.[18] found significantly higher mean WBC and NLR values and a lower lymphocyte count in their severe ICP group than in controls, and NLR was found to be even higher in patients with mild ICP. They also found a significant association between fasting TBA levels and NLR[18]. However, we found that neutrophil and lymphocyte counts were significantly decreased in patients with ICP; NLRs were similar between the normal and ICP group. Platelets in the blood vary in size, with the granules and adhesion molecules of the platelets increasing when they become larger and play an active role in homeostasis[34]. MPV, which is the most frequently used platelet size measurement, is also an index of platelet activation[34]. Platelets release thrombin, which plays a role in inflammation[34] and angiogenesis[35], and a high platelet volume allows greater coagulability and fibrinolysis[36]. However, there is a limited number of studies about the relationship between MPV and the severity of ICP, and the relationship between MPV and perinatal outcomes, even though an MPV increase can be seen in patients with ICP[31,32]. Kebapcilar et al.[31] investigated the relationship between coagulation parameters and low 5-minute Apgar scores in both patients with ICP and normal pregnancies. In addition, Oztas et al.[32] reported higher MPVs in patients with ICP when compared with a control group, with an increased preterm delivery likelihood just after exceeding an MPV of 11.2 fL. In our study, MPV was significantly increased in women with ICP cases compared with healthy controls. Moreover, apart from the PLR and WBC, the MPV levels increased in the severe group.

Study Limitations

The main limitation of this study was its retrospective design, but despite this methodologic limitation, this research investigated all of the available hematologic inflammatory parameters. It confirmed prior data that inflammatory markers are significantly increased in patients with ICP. Despite the significant changes in the inflammatory markers in patients with ICP, only MPV was significantly increased with the severity of the disease. These results suggest that MPV may be a valuable marker in patients with severe ICP, but large scale studies are needed to confirm this result.

Conclusion

Based on the results of this study, the inflammatory markers were significantly increased in patients with ICP. PLR, WBC, and MPV were all significantly increased, whereas the RDW was significantly decreased in ICP. MPV was related to the severity of disease and might be a valuable marker for ICP disease severity in the future.

Ethics

Ethics Committee Approval: This study was approved by the Zeynep Kamil Women and Children's Health Training and Research Hospital Local Ethics Committee (approval number: 136).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ç.Y.A., F.V., O.P., Ç.K., Concept: Ç.Y.A., F.V., Design: Ç.Y.A., F.V., Data Collection or Processing: Ç.Y.A., E.B.E., İ.Y., A.E., O.P., Analysis or Interpretation: Ç.Y.A., F.V., E.B.E., Literature Search: Ç.Y.A., İ.Y., Ç.K., Writing: Ç.Y.A., F.V.

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

Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.87864

Turk J Obstet Gynecol 2017;14:166-9

Third trimester anemia extends the length of hospital stay after delivery

Üçüncü trimesterdeki anemi doğum sonrası hastanede kalış süresini arttırmaktadır

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Abstract

Objective: To assess the relationship between maternal third trimester anemia and hospital stay after delivery.
Materials and Methods: In this retrospective cross-sectional study, 695 women aged 18-42 years were included between January 2016 and June 2016. Obstetric outcomes and fetal outcomes were measured. Statistical analysis was performed using SPSS, version 19.0 (SPSS, Chicago, Illinois).
Results: The prevalence of anemia in this study was 15.2%. The study population was divided into three groups according to hemoglobin (Hb) levels. Group 1 consisted of patients with Hb <8.5 g/dL, group 2 Hb 8.5-11 g/dL, and group 3 Hb >11 g/dL. Higher levels of Hb were associated with shorter stay in hospital (p=0.028). In binary comparison, no significant difference was observed between groups 2 and 3, whereas it was statistically different from group 1. Fetal weight (p=0.562), neonatal intensive care unit admission (p=0.596), APGAR score 1st (p=0.674) and 5th minute (p=0.876), type of delivery (p=0.831), and gestational age (p=0.798) were not statistically different between the groups; however, hospitalization time was significantly different (p=0.028).
Conclusion: Maternal anemia in the third trimester prolongs hospitalization time after delivery. Anemia effects pregnancy and the fetus in the postpartum period in addition to the prenatal period.
Keywords: Anemia, hospitalization, third trimester

Öz

Amaç: Üçüncü trimester anemisi ile doğum sonrası hastanede yatış süresi arasındaki ilişkinin değerlendirilmesidir.
Gereç ve Yöntemler: Bu retrospektif kesitsel çalışmaya, 18-42 yaşları arasında 695 kadın, Ocak 2016 ve Haziran 2016 tarihleri arasında dahil edildi. Obstetrik ve fetal sonuçlar değerlendirildi. İstatistiksel analiz, SPSS 19,0 versiyonu kullanılarak yapıldı (SPSS, Chicago, Illinois).
Bulgular: Bu çalışmadaki anemi prevalansı %15,2 olarak bulundu. Çalışma popülasyonu hemoglobin (Hb) değerlerine göre üç gruba ayrıldı. Grup 1'e Hb değerleri 8,5 altında olan hastalar, grup 2'ye Hb değeri 8,5-11 arasında olan hastalar, grup 3'e ise Hb değeri 11'in üzerinde olan hastalar dahil edildi. Hb değerlerinin yüksek olması hastanede kalış süresini kısaltmaktadır (p=0,028). İkili karşılaştırmalarda grup 2 ve 3 arasında anlamlı fark saptanmazken, grup 1 ile diğer iki grup arasında anlamlı fark saptanmıştır. Fetal ağırlık (p=0,562), yenidoğan yoğunbakım yatışı (p=0,596), APGAR 1. dakika (p=0,674), 5. dakika değeri (p=0,876), doğum şekli (p=0,831) ve doğum zamanı (p=0,798) arasında istatistiksel olarak anlamlı fark saptanmadı, ancak hastanede kalma süresi açısından karşılaştırıldığı gruplar arasında anlamlı fark saptandı (p=0,028).
Sonuç: Üçüncü trimesterde maternal anemi doğumdan sonra hastanede yatış süresini uzatmaktadır. Anemi gebelik ve fetüsü prenatal dönemin yanı sıra postnatal dönemde de etkilemektedir.
Anahtar Kelimeler: Anemi, hastanede kalış süresi, üçüncü trimester

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PRECIS: In this study, we have assessed the relationship between maternal third trimester anemia and hospital stay after delivery.

Introduction

Maternal anemia remains an important public health problem in under-developed and developing countries like ours. The Centers for Disease Control and Prevention suggested that anemia should be defined as hemoglobin levels of less than 11 g/dL (hematocrit less than 33%) in the first and third trimesters and less than 10.5 g/dL (hematocrit less than 32%) in the second trimester[1]. The prevalence of anemia among pregnant women seems to differ between countries and even between regions of a country. It is estimated that half of all pregnant worldwide women are anemic[2]. The anemia prevalence in different regions of Turkey were reported between 27% and 88%, on an average 50%[3].

Anemia is a physiologic result of pregnancy, and to some extent, it may be necessary. The absence of physiologic anemia has been suggested to be associated with stillbirth[4,5]. Maternal anemia may have a significant impact on fetal outcome in terms of preterm birth and infant deaths[6]. The relationship between low birth weight, preterm birth, small for gestational age, perinatal mortality, neonatal mortality, gestational diabetes, preeclampsia, and mode of delivery according to maternal anemia status has been shown in the literature, yet it is not precisely concluded[4]. However, the impact of severity of anemia on obstetric outcomes is controversial. Several studies suggest that only severe anemia leads to poor obstetrics outcomes[7].

The aim of our study was to identify the impact of third trimester hemoglobin values on perinatal outcomes and type and time of delivery.

Materials and Methods

The retrospective cross-sectional study was conducted in the obstetrics out-patient clinic in a university-based hospital between January 2016 and April 2016. All data of women who were referred for routine third trimester follow-up were recorded. The inclusion criteria were as follows: age between 18 and 45 years, singleton pregnancy, iron supplementation started around 18th gestational week, gestational age between 28 and 40 weeks during the study period, and at least one complete blood count during the third trimester. The exclusion criteria were history of preterm delivery, elevated risk pregnancy, previous uterine surgery including cesarean section, multiple gestation, any type of chronic systemic disease, and not receiving iron supplementation.

All analyzed data were obtained from the patients' charts and medical records. Patients were divided into three groups: group 1 consisted of patients with Hb <8.5 g/dL, group 2 comprised patients with Hb 8.5- 11 g/dL, and group 3 constituted patients with Hb >11 g/dL. The main outcome measures were perinatal and neonatal outcome parameters such as gestational age at delivery, mode of delivery, birth weight and APGAR scores, type of delivery, and duration of hospitalization. Ethics committee approval and informed consent were not taken as it is a retrospective study.

Statistical Analysis

Statistical analysis was performed using SPSS, version 19.0 (SPSS, Chicago, Illinois). Due to the normal distribution of all data, ANOVA test was used for the determination of differences between the three groups. The independent sample t-test was used for to determine differences between the two sexes. Categorical data were assessed using the chi-square test. $P<0.05$ was considered statistically significant.

Results

A total of 695 pregnant women were assessed for eligibility. Among those, 265 patients were excluded due to being lost to follow-up (n=97), multiple pregnancy (n=13), not receiving iron supplementation (n=92), presence of chronic disease (n=73). As a result, 433 patients were included in the final analyses. The mean third trimester hemoglobin level was 11.98±5.44 mg/dL (7-15.2 mg/dL). Anemia prevalence in our study population was 15.2%. The characteristics of the study population are presented in Table 1.

Table 2 represents the comparison of perinatal outcome measures between groups 1, 2, and 3. The only parameter that showed a statistically significant difference between the groups was the duration of hospitalization. The duration of hospitalization was significantly longer in group 1 than in groups 2 and 3 (p=0.028).

Discussion

The aim of our study was to identify the impact of third trimester hemoglobin values on perinatal outcomes and type and time of delivery. According to the results, severe anemia during the third trimester results in increased duration of hospitalization. Otherwise, it has no adverse effects on outcomes.

Anemia during the third trimester of pregnancy is defined as Hb less than 11 g/dL or hematocrit less than 33% by the CDC[1] and its prevalence can differ between populations and

Table 1. Characteristics of the study population

	Mean ± SD	Minimum	Maximum
Age (year)	28.80±5.36	18	42
BMI (kg/m^2)	27.2±3.21	22	31
Gestational age (weeks)	38±2.31	27	41
Parity	1.39±0.93	1	4
Hemoglobin (mg/dL)	11.98±1.35	7	15.2

SD: Standard deviation, BMI: Body mass index

Table 2. Comparison of hemoglobin values between the three groups (maternal third trimester hemoglobin level groups as lower 8.5 mg/dL, between 8.5 and 11 mg/dL, upper 11 mg/dL)

	Group 1 (n=24)	Group 2 (n=87)	Group 3 (n=322)	Significance p
Birth weight, grams[a]	3357.5±256	3203.7±152	3110.4±203	0.562
Newborn hospitalization, n (%)[b]	0 (0)	7 (8.07)	18 (5.59)	0.596
Gestational age at birth, weeks[a]	38.2±6	38.3±4	38.5±2	0.798
APGAR 1st minute[a]	7.6±1.5	7.7±.2.14	7.5±1.78	0.510
APGAR 5th minute[a]	9.2±0.23	9.1±0.14	9.0±0.71	0.876
Mode of delivery, n (%)[b]				
Vaginal[b]	13 (54.1)	46 (52.8)	172 (53.4)	0.831
Cesarean section[b]	11 (45.8)	43 (47.1)	150 (46.5)	
Hospital stay, days[a]	5.2±1.2	2.5±1.1	2.8±1.4	0.028*

*The significance stems from the differences between group 1 and 2 (p=0.024) and group 1 and group 3 (p=0.043) ([a]Compared using one-way ANOVA test, [b]Compared using chi-square test)

geographic regions. In our study, the prevalence of anemia was found as 15.2% during the third trimester of pregnancy. Although different anemia prevalences have been reported from various parts of our country, the prevalence of third trimester anemia in our study was similar to that reported from a large maternity hospital in our city[7]. Both the prevalence from our study and the other study seem to be lower than other parts of the country. This situation might be due to higher socioeconomic status resulting in appropriate follow-up during pregnancy and proper iron replacement therapy. Furthermore, grand-multiparity was rare in our study population and multiparity is also a risk factor for anemia in pregnant women[8].

According to the results obtained from our study, severe third trimester anemia is associated with longer hospital stay after delivery. The main reason for longer hospitalization is postpartum anemia treatment, either by transfusion or intravenous iron supplementation[9]. Lin et al.[10] reported that every 1-U decrease in hemoglobin level extended the median length of hospital stay by 1.5 days among anemic general medical inpatients. Also, it has been reported that anemia increased the 30-day unplanned readmission rate in addition to extending hospital stay[11].

Iron supplementation may be preventive for adverse effects of anemia[12,13]. A recent Cochrane review Peña-Rosas et al.[13] concluded that women with anemia were at greater risk of having low-birth-weight infants. On the contrary, Scholl et al.[14] suggested a subdivision of anemia according to etiology and gestational age and reported that third trimester anemia due to iron deficiency had no correlation with low birth weight. In addition, some other studies concluded that after adjusting for confounders, anemia was not relevant to poor neonatal outcomes because these relationships were a result of poverty or low socioeconomic status[14]. In our study, we also failed to show a significant impact of anemia on neonatal outcomes such as birth weight, APGAR scores at first and fifth minutes, and neonatal intensive care unit admission. As local clinical policy, we clamp the cord 1-3 minutes after birth and this may be the reason for the neonatal results obtained from our study.

Sehgal et al.[15] reported that anemia did not affect the mode of delivery in nulliparous pregnant women. Mild anemia seems to be inconclusive for estimating mode of delivery[15]. Van Bogaert[16] also suggested no effect of anemia during first trimester of pregnancy on the mode of delivery. Our results were also in accordance with these studies. In the present study, we excluded patients with previous uterine surgery to identify the effect of anemia on the mode of delivery. However, we found no significant effect of anemia on the mode of delivery. In addition, the indications for cesarean section did not differ between the groups.

One of the strengths of our study was the standardized follow-up and management protocol for each patient. Also, neonatal results were obtained from a single center. The strict inclusion and exclusion criteria to create a homogenous study population add credence to our results.

Study Limitations

The main limitations of our study were the retrospective design and small number of patients included. However, the present study was conducted in a tertiary referral center and we excluded high-risk patients. Hence, it was not possible to include more patients in such a study using strict inclusion criteria, conducted in a single center.

Conclusion

Anemia during the third trimester extends the length of hospital stay in the postpartum period. However, it does not seem to affect neonatal outcomes and mode of delivery. Prevention and/or treatment of anemia before delivery can shorten the duration of hospital stay. Prospective studies in large cohorts are needed to clarify the exact effects of mild and severe anemia on perinatal and neonatal outcomes.

Ethics

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

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.87404

Turk J Obstet Gynecol 2017;14:170-5

Comparison of bupivacaine and ropivacaine in combination with fentanyl used for walking epidural anesthesia in labor

Yürüyen epidural anestezide fentanil ile kombine kullanılan bupivakain ve ropivakainin karşılaştırılması

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Abstract

Objective: Effective pain relief during labor is essential to reduce maternal and perinatal morbidity arising due to pain-induced maternal sympathetic activation, and to avoid unnecessary cesarean sections performed due to maternal anxiety. Walking epidural analgesia on labor reveals lower pain scores, leading to higher maternal satisfaction with better cardiovascular and pulmonary physiology. Despite the extensive use and relative safety of bupivacaine, newer drugs such as ropivacaine have been developed as alternative agents to decrease the risk for cardiac and central nervous system toxicity.

Materials and Methods: One hundred women who requested epidural analgesia in active labor were randomly allocated into two groups; one group received 20 mL of ropivacaine 0.125% with fentanyl 50 µg and the other received 20 mL of bupivacaine 0.125% with fentanyl 50 µg. The efficacy of analgesia, adverse effects, and obstetric and neonatal outcomes of both groups were compared.

Results: There were no differences between the two study groups in the measured obstetric and neonatal outcomes. The onset time, duration of analgesia, and sensory levels were similar between the groups. Visual analog pain scale scores did not differ between the groups before analgesia or at any of the subsequent evaluation periods.

Conclusion: Both ropivacaine and bupivacaine provide equivalent labor analgesia with high maternal satisfaction and tolerable adverse effects in the clinically used dose range. No adverse obstetric or neonatal outcomes were observed in either group. Therefore, either drug is a reasonable choice for labor analgesia and can be used without jeopardizing the safety of the mother and fetus.

Keywords: Bupivacaine, epidural, fentanyl, labor, ropivacaine

Öz

Amaç: Doğum sırasında etkin ağrı giderimi, ağrının neden olduğu maternal sempatik aktivasyon nedeniyle ortaya çıkan maternal ve perinatal morbiditeyi ve anne kaygısı nedeniyle uygulanan gereksiz sezaryen oranlarını azaltmak için önemlidir. Doğumda yürüyen epidural analjezi uygulanması daha iyi kardiyovasküler ve pulmoner fizyoloji ile anne memnuniyetine yol açan daha düşük ağrı skorları ortaya koyar. Bupivakainin yaygın kullanımı ve görece emniyetine rağmen, ropivakain gibi yeni ilaçlar, kardiyak ve merkezi sinir sistemi toksisitesi riskini azaltmak için alternatif ajanlar olarak geliştirilmiştir.

Gereç ve Yöntemler: Aktif doğumda epidural analjezi isteyen 100 kadın randomize olarak iki gruba ayrıldı; bir gruba 20 mL ropivakain %0,125 + fentanil 50 µg ve diğer gruba 20 mL %0,125 bupivakain ile fentanil 50 µg verildi. Her iki grupta analjezi etkinliği, yan etkiler, obstetrik ve neonatal sonuçlar karşılaştırıldı.

Bulgular: Obstetrik ve neonatal sonuçlar açısından iki çalışma grubu arasında fark yoktu. Başlangıç yaşı, analjezi süreleri ve duyu seviyeleri gruplar arasında benzerdi. Görsel analog ağrı skalası skorları, analjezi öncesi veya sonraki değerlendirme periyodlarının herhangi birinde gruplar arasında fark göstermedi.

Sonuç: Hem ropivakain hem de bupivakain, klinik olarak kullanılan doz aralığında yüksek anne memnuniyeti ve tolere edilebilen yan etkiler ile eşit analjezik etkinlik sağlamıştır. Her iki grupta istenmeyen obstetrik ve neonatal sonuç gözlenmemiştir. Bu nedenle, anne ve fetüsün güvenliğini tehlikeye atmadan, her iki ilaç da doğum analjezisi için makul bir seçenek olarak değerlendirilebilir.

Keywords: Bupivakain, epidural, fentanil, doğum, ropivakain

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Received/Geliş Tarihi: 05.06.2017 **Accepted/Kabul Tarihi:** 21.06.2017

PRECIS: Ropivacaine and bupivacaine seem to be equiopotent at clinically used concentrations and can both be reasonable choices for labor analgesia.

Introduction

Labor pain is reported to be one of the most severe pains that have ever been evaluated[1,2]. In a previous study, 41% of women considered it as the worst experience that they had ever had. Fear of labor pain seems one of the most important reasons for the tendency to cesarean section[2]. Additionally, pain-induced maternal sympathetic activation in labor compromises fetal oxygenation. Therefore, effective pain relief during labor is essential to reduce maternal and perinatal morbidity and to avoid unnecessary cesarean sections performed due to maternal anxiety[3].

Walking epidural analgesia on labor reveals lower pain scores, leading to higher maternal satisfaction with better cardiovascular and pulmonary physiology[3]. The ideal drugs to be used for labor analgesia should have a long duration of action with minimum motor blockade, limited placental transfer, and no significant adverse effects on the mother and fetus[4,5]. Bupivacaine is the most commonly used drug for this purpose. Despite the extensive use and relative safety of bupivacaine, newer drugs such as ropivacaine and levobupivacaine have been developed as alternative agents to decrease the risk for cardiac and central nervous system toxicity. Another advantage of these drugs is less motor blockade compared with bupivacaine[6]. The addition of opioids to these local anesthetics such as sufentanil or fentanyl is preferable due to their dose minimizing and adverse-effect-reducing properties[7]. The purpose of the current study was to compare the effects on obstetric and neonatal outcomes between ropivacaine and bupivacaine in combination with fentanyl used in walking epidural analgesia.

Materials and Methods

This prospective randomized controlled trial was conducted at a tertiary center during a one-year period. The study was approved by the Cerrahpaşa University Local Ethics Committee (approval number: P20/1999). Written consent for participation was obtained prior to recruitment into the study.

Women aged 18-35 years, classified as American Society of Anesthesiologists score I and II who requested epidural analgesia in active labor with cervical dilatation 3-4 cm, and uterine contractions ≥3/10 minutes between 37-41 weeks' gestational age with a singleton pregnancy in the vertex position were enrolled in this study.

Women with high risk pregnancies as defined by the obstetrician such as severe preeclampsia, insulin-dependent diabetes mellitus, multiple pregnancies or with any contraindications to epidural techniques such as coagulopathies, spinal deformities, local infections, and any sensitivity to the drug were excluded.

The patients were randomized 1:1 to each treatment arm, with stratification based on parity. One hundred participants who met the above mentioned criteria were allocated into two groups. Group R received 20 mL of ropivacaine 0.125% with fentanyl 50 µg, and group B received 20 mL of bupivacaine 0.125% with fentanyl 50 µg.

No sedative premedication was given to the participants. After intravenous prehydration with 500 mL 0.09% NaCl solution, a 16-gauge Touhy needle was placed in the patients at the level of L3-4 or L4-5 interspaces via a midline approach under complete aseptic conditions. The loss of resistance technique was used to identify the epidural space. After monitoring any aspirate of blood or cerebrospinal fluid via the catheter, a 3 mL test dose of the study medication was administered. If there were no signs of an intravascular or intrathecal injection for the following 5 minutes, the remaining dose of the selected medication was administered. The catheter was inserted about 3-4 cm into the epidural space and securely fixed. After the insertion, patients were placed in the supine position with left uterine displacement.

Vital parameters of the mother such as heart rate, blood pressure, respiratory rate, and maternal saturation were recorded before and every 15 minutes after the injection. Onset of analgesia was evaluated as the time after injection until the first painless contraction occurred. The effectiveness of the epidural block was evaluated using a visual analog pain scale (VAS) (VAS: 0 to 10, with 0 being no pain and 10 being the worst imaginable pain). An additional dose of 5 mL of the analgesic solution was injected whenever the parturient had VAS ≥3 during labor. The sensory level was assessed using the pinprick method. Preservation of motor function was determined using the modified Bromage scale in both legs (0: no paralysis, full flexion of knees and feet, 1: inability to raise the extended leg and ability to move knees and feet; 2: inability to move knees but ability to move feet; 3: inability to flex ankle joints, complete motor blockade of lower limbs). Maternal adverse effects during the procedure such as nausea, vomiting, pruritus, bradycardia, trembling, and hypotension were recorded.

Fetal wellbeing and uterine contractions were monitored using cardiotochography. For the comparison of uterine activity, a 30-minute postinjection period was taken into account. The duration of the first and second stages of labor, and mode of delivery were recorded. Neonatal welfare was assessed using Apgar scores at 1 and 5 minutes. Maternal satisfaction about labor analgesia was determined after 24 hours on a four-point scale.

Statistical Analysis

Data were analyzed using IBM SPSS 22.0 software (SPSS Inc., IBM, Chicago, Illinois, USA), and descriptive data are expressed as mean ± standard deviations and frequencies. The Mann-Whitney U test, Student's t-test, and chi-square test were

used for comparisons. A probability (p) value of <0.05 was considered significant.

Results

The enrolled 100 women were assigned to either the ropivacaine group (group R) (n=50) or the bupivacaine group (group B) (n=50). The demographic characteristics were similar between the two groups. Maternal and fetal hemodynamic data were also comparable (Table 1).

Maternal adverse effects (nausea and pruritus) were seen in both groups (group B: 20%, group R: 10%; group B: 10%, group R: 20%, respectively). Trembling was only seen in two patients of group R. There were no cases of motor blockade in either group.

The onset time, duration of analgesia, and sensory levels were similar between the groups. VAS scores did not differ between the groups before analgesia or at any of the subsequent evaluation periods. Ten parturients in group R and 11 in group B required an additional bolus of 5 mL after 2-3 hours (Table 2).

Maternal satisfaction with labor analgesia was mostly defined as excellent in both groups and no significant difference was observed between the groups (Table 2).

Obstetric characteristics and outcomes are shown in Table 3. Four parturients in each group required cesarean section and one parturient required forceps application in group B. No significant difference was found between the groups when assessed for uterine activity.

Twenty percent of patients in group B and 28% in group R required local anesthesia for closure of the episiotomy wound.

There were no differences between the two study groups in the measured neonatal outcomes (Table 3).

Table 1. Demographic characteristics of the patients and data of maternal and fetal hemodynamic parameters

	Ropivacaine (n=50)	Bupivacaine (n=50)	p
Age (year)	23.62±3.86	22.58±3.03	0.695
Height (cm)	161.9±4.86	162.86±4.11	0.288
Weight (kg)	64.06±6.96	63.9±5.67	0.515
Parity (n)			-
Primiparae	32	32	
Multiparae	18	18	
ASA group (n)			-
I	34	40	
II	16	10	
Maternal heart rate			
Before analgesia	89.3±4.6	90.1±5.8	0.784
Fifteen minutes after injection	81.4±5.9	80.2±6.9	0.685
Thirty minutes after injection	86.1±7.4	84.5±6.4	0.832
Maternal respiratory rate			
Before analgesia	16.18±0.74	16.22±0.61	0.771
Fifteen minutes after injection	12.24±0.47	12.16±0.37	0.351
Thirty minutes after injection	12.08±0.37	12.22±0.41	0.450
Maternal systolic blood pressure			
Before analgesia	116.76±9.65	117.94±8.37	0.515
Fifteen minutes after injection	104.06±9.53	104.96±9.39	0.635
Thirty minutes after injection	110.50±8.22	112.20±8.64	0.316
Maternal diastolic blood pressure			
Before analgesia	74.40±6.03	74.28±5.80	0.919
Fifteen minutes after injection	70.24±5.98	70.18±6.07	0.960
Thirty minutes after injection	74.47±6.11	73.36±5.77	0.912
Fetal heart rate			
Before analgesia	143.02±12.59	144.68±10.37	0.474
Fifteen minutes after injection	139.22±15.68	139.62±16.19	0.900
Thirty minutes after injection	139.86±10.32	142.02±9.88	0.288

Data are given as mean ± standard deviations or frequencies
ASA: American Society of Anesthesiologists

Discussion

Epidural analgesia has become a widely-used technique for providing pain relief in labor. Nowadays, there is an increase in the number of the epidural drugs. The most recent literature focuses on new enantiomers such as ropivacaine, which have reduced risk of cardiotoxicity compared with bupivacaine[7]. In our comparison of these two agents in the present study, no motor blockade was observed and maternal satisfaction rates were similar with tolerable adverse effects. In addition, no obstetric or neonatal adverse effects were observed.

Some previous studies claimed that epidurals prolonged labor, and increased oxytocin requirements and instrumental and operative delivery rates[8,9]. This was explained as motor block in perineal and abdominal muscles caused by epidural local anesthetics, which may cause abnormal internal rotation of the fetal head leading to dystocia[9].

In a meta-analysis, it was suggested that the type of epidural analgesia might influence spontaneous vaginal delivery rates. Analgesia combined with low-dose opioid and local anesthetic has been asserted to result in lower rates of instrumental deliveries[10,11]. Some investigators suggested that ropivacaine was associated with an increased rate of spontaneous vaginal delivery compared with bupivacaine due to a reduction in motor block[12]. Lv et al.[7] reported in their meta-analysis of 10 impact studies that ropivacaine was associated with less motor blockade but a higher incidence of instrumental delivery. Halpern et al.[13] showed that the rate of motor block was more frequent in the bupivacaine group but the incidence of spontaneous vaginal delivery was similar regardless of whether ropivacaine or bupivacaine were used for labor analgesia. There are conflicting results in the literature in the comparison of these two local anesthetics regarding the mode of delivery. In the current study, the vaginal spontaneous labor rate was high and there was no significant difference between the groups in regard to operative delivery.

It is assumed that ropivacaine has a greater selectivity for sensory fibers than motor fibers due to its lower lipophilic capacity compared with bupivacaine. Accordingly, it is less likely to cause motor blockade and neurotoxicity[4,6]. There were no cases of motor blockade in either group in our study. This could be related to the use of very low and titrated concentrations of a local anesthetic through the addition of opioids. It may also account for our high spontaneous vaginal delivery rate. Higher concentrations of local anesthetic may be the reason of increased motor blockade and instrumental delivery rates in previous studies.

Lee at al.[14] reported that bupivacaine was associated with prolongation in the first stage of labor. This may result from higher concentrations of initiated analgesia with a 0.25% solution, which triggers motor block, leading to elongation of labor. In contrast, other comparative studies using these local anesthetics in a range of 0.075-0.125% found no differences in the durations of the first or second stages of labor, similar to our results[15,16].

Our findings regarding neonatal outcomes were comparable with the literature[4,13-16]. There were no significant differences in the indicators of neonatal wellbeing between the two groups. In a study conducted by Writer et al.[12], lower neurologic and adaptive capacity scores with bupivacaine versus ropivacaine were found. We did not assess this outcome due to the conflicting results about its reliability in newborn evaluations[17].

Table 2. Effectiveness of analgesics in both groups and pain assessment using the 0-10 visual analogue scale

	Ropivacaine (n=50)	Bupivacaine (n=50)	p
The onset time of analgesia (minute)	11.18±1.41	11.54±2.21	0.335
The duration of analgesia (minute)	123.56±19.45	130.30±19.65	0.478
Initial pain score before injection	8.30±0.67	8.12±0.62	0.171
Fifteen minutes after injection	0.42±0.92	0.20±0.80	0.209
Thirty minutes after injection	0.06±0.24	0.08±0.34	0.735
One hour after injection	0.04±0.19	0.02±0.14	0.562
Two hours after injection	0.38±0.72	0.30±0.61	0.553
Three hours after injection	4.14±1.06	3.96±0.75	0.333
Need for additional dose (%)	20	22	0.120
Maternal satisfaction of patients for labor analgesia (n)			
Excellent	40	39	
Good	8	8	
Unsatisfactory	2	2	
Terrible	-	1	

Data are given as mean ± standard deviations or percentages

Table 3. Obstetric characteristics and data of obstetric and neonatal outcomes

	Ropivacaine (n=50)	Bupivacaine (n=50)	p
Gestational weeks	39.42±0.60	39.60±0.90	0.195
Initial cervical dilatation (cm)	4.66±0.49	4.47±0.57	0.081
Initial cervical effacement (%)	68.70±8.31	66.50±9.16	0.212
Duration of first stage (minute)	130.31±60.60	150.93±100.55	0.227
Duration of second stage (minute)	35.20±9.00	38.22±13.10	0.192
Duration of labor (minute)	165.52±63.20	189.16±106.37	0.189
Need for oxytocin augmentation (%)	72	80	0.348
Mode of delivery (%)			
Normal vaginal delivery	92	90	0.726
Instrumental delivery	0	2	0.314
Cesarean section	8	8	0.999
Need for episiotomy (%)	56	60	0.685
The number of uterine contractions[a]	4.46±0.86	4.48±0.88	0.909
The duration of uterine contractions[a] (second)	68.40±19.72	66.10±19.25	0.557
Montevideo unit[a]	208.40±56.33	197.60±57.55	0.345
Apgar score			
At 1 minute	8.35±0.93	8.10±1.09	0.232
At 5 minute	9.50±0.68	9.22±0.72	0.062
Abnormal arterial blood gases	2	4	0.557
Required mask ventilation	10	12	0.626
Incidence of respiratory distress	4	2	0.557
Required tracheal intubations	0	0	-
Required NICU admission	4	2	0.557

Data are given as mean ± standard deviations or percentages
[a]Assessment of uterine activity in a 30-minute postinjection period
NICU: Neonatal intensive care unit

Shokry et al.[18] compared two groups receiving 0.125% bupivacaine and 0.2% ropivacaine, each with fentanyl 100 µg and found an non-significant faster onset of action and significantly shorter duration of analgesia in the ropivacaine group. In contrast, Chora and Hussain[4] showed significantly faster onset of analgesia in the bupivacaine group and longer duration in the ropivacaine group. Unlike these, the onset and duration of analgesia for both groups was comparable in current study, consistent with the research of Beilin et al.[19]. Bawdane et al.[20] recorded similar pain scores, sensory levels, and overall maternal satisfaction between the two groups, as we observed. Although ropivacaine is suggested to be less potent than bupivacaine[21], they appear to be equipotent at clinically used concentrations.

Study Limitations

The limitation of the current study is its small sample size in both groups, further research should be organized with large sample groups.

Conclusion

Overall, both ropivacaine and bupivacaine can provide equivalent labor analgesia with high maternal satisfaction and tolerable adverse effects in the clinically used dose range. A combination with opioids is preferable considering their dose lowering effect. No adverse obstetric or neonatal outcomes were observed in either group in the current study. Therefore, from a clinical perspective, either drug is a reasonable choice for labor analgesia and can be used without jeopardizing the safety of the mother and fetus.

Ethics

Ethics Committee Approval: The study was approved by the Cerrahpaşa University Local Ethics Committee (approval number: P20/1999).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.06337

Turk J Obstet Gynecol 2017;14:176-80

Anemia prevalence at the time of pregnancy detection

Gebeliğin tanısı ile birlikte saptanan anemi prevalansı

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Abstract

Objective: Anemia in the first trimester of pregnancy is the situation as described by the World Health Organization when the level of hemoglobin (Hb) is less than 11 g in 100 cc of blood. The prevalence of this problem is 18% in developed countries, whereas it is between 35-75% in developing countries. In this study, we aimed to determine the prevalence of anemia at the time of pregnancy detection.

Materials and Methods: A retrospective cross-sectional study was designed to determine the prevalence of anemia. A total of 5228 first trimester pregnant women were admitted to the study between 2012 and 2014. Hb levels of 11 to 9.5 g/dL, 9.5 to 8 g/dL, and less than 8 g/dL were considered as mild, moderate, and severe anemia, respectively.

Results: We detected mild, modarate, and severe anemia at rates of 16.64%, 3.07%, and 0.28%, respectively, in our population. The overall prevalence of anemia at the time of detection of pregnancy was 20.0%.

Conclusion: Anemia is a significant risk factor for maternal mortality in developing countries. The prevalence of anemia at the time of pregnancy detection was 20% and this rate is close to those indicated in developed countries.

Keywords: Pregnancy, anemia, prevalence

Öz

Amaç: Gebelikte ilk trimesterde anemi, hemoglobin (Hb) düzeyinin 100 cc kanda 11 g'den daha düşük olduğu durumdur. Bu problemin görülme prevalansı gelişmiş ülkelerde (%18) ve gelişmekte olan ülkelerde (%35-75) olarak bildirilmiştir. Bu çalışma ile, gebe olan popülasyonda gebelik saptandığı birinci trimesterde mevcut anemi prevalansını hesaplamayı amaçladık.

Gereç ve Yöntemler: 2012-2014 yılları arasında adet gecikmesi nedeniyle başvuran ve gebelik tanısı konulan 5228 gebe kadında, anemi prevalansının saptanması için düzenlenmiş retrospektif kesitsel tipte bir araştırmadır. Hb değeri 11-9,5 mg/dL hafif, 9,5-8 mg/dL orta ve 8 mg/dL altıda ağır anemi olarak kabul edildi.

Bulgular: Gebe popülasyonumuzda gebelik tespit edildiğinde hafif anemisi olan gebelerin oranı %16,64'tür. Orta anemi olan gebe oranı ise %3,07'dir. Hb değeri 8 mg/dL altında olan ağır anemi ise %0,28'dir. Genel popülasyonda gebelik tespitinde anemi prevalansı ise %20,0'dır.

Sonuç: Anemi gelişmekte olan ülkelerde maternal mortalite için önemli bir risk faktörüdür. Gebelik saptandığı birinci trimesterde mevcut anemi prevalansını %20 saptadık ve bu gelişmiş ülkelerin oranına yakın görünmektedir.

Anahtar Kelimeler: Gebelik, anemi, prevalans

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Received/Geliş Tarihi: 13.03.2017 **Accepted/Kabul Tarihi:** 09.08.2017

PRECIS: The prevalence of anemia at the time of pregnancy detection was 20% and this is close to the rates of developed countries.

Introduction

It has been demonstrated that anemia in pregnancy is one of the main health problems and affects the results of pregnancy negatively[1]. The prevalence of anemia is still in question in our country[2].

Anemia can be classified as acquired or hereditary. Deficiency anemia (iron, folate, and vitamin B12), anemias depending on blood loss, chronic disease anemias, acquired hemolytic anemias, and aplastic anemia can be considered as acquired anemias, whereas sickle cell anemia, thalassemia, and Fanconi anemias are considered as hereditary anemias[3]. Iron-deficiency anemias (IDA) are responsible for more than half of all cases in all regions worldwide (where malaria is not an endemic). In pregnancy, the most frequently encountered anemia is IDA[4].

Anemia in the 1st and 3rd trimester of pregnancy was defined by the Centers for Disease Control and Prevention in 1989 as hemoglobin (Hb) or hemotocrit less than 11 g/dL or 33%, respectively, and when the level of Hb or hemotocrit is less than 10.5 g/dL or 32%, respectively, in the 2nd trimester of pregnancy[1]. According to the World Health Organization (WHO), anemia in pregnancy in any trimester is considered when the level of Hb in less than 11 g/dL[4]. This definition was made in 2001 and is still valid today. When the level of Hb is less than 7 g/dL during pregnancy, it is considered as severe anemia and medical treatment is required. It has been revealed that anemia observed in the first trimester of pregnancy enhances the possibility of premature birth and low birth weight, as well as low APGAR scores[5,6].

In our study, we aimed to identify the prevalence of anemia in patients at the time of pregnancy detection.

Materials and Methods

This study is a retrospective cross sectional study that was designed to detect anemia prevalence of 5228 pregnant women who presented because of delayed menstrual periods and were diagnosed as being pregnant between 2012 and 2014. Hb levels of 11 to 9.5 g/dL, from 9.5 to 8 g/dL and less than 8 g/dL were considered as mild, moderate, and severe anemia, respectively[7,8]. Pregnants were classified into 4 groups according to their Hb levels as follows; group 1: severe anemia, group 2: moderate anemia, group 3 mild anemia, and group 4 as normal (Hb levels 11 g/dL or higher).

The complete blood count of the women was measured using an automated blood analyzer (Beckman-Coulter, USA). The three-year results were evaluated and classified according to their Hb levels. We did not report the risk factors and independent predictors of anemia. This study was approved by the Etimesgut Military Hospital Local Ethics Committee (approval number 8000-11-12) and all women who accepted to take part gave written informed consent before enrollment in the study.

Statistical Analysis

The collected data were analyzed using the Statistical Package for Social Sciences version 14.0 (SPSS Inc., Chicago, USA). Continuous variables are expressed as mean ± standard deviation, whereas categorical variables are denoted as numbers or percentages where appropriate.

Results

Groups 1, 2, 3, and 4 comprised 15, 161, 870, and 4182 patients, respectively. The average age was 30.2±4.75 years and the average Hb was 11.8±1.15 g/dL in our population (Table 1) (Figure 1).

In groups 1, 2, 3, and 4, the average ages were 30.4±7.14, 30.2±5.42, 30.1±4.4, 30.2±4.7 years, respectively. The respective Hb levels in each group were 7.5±0.35, 9.0±0.34, 10.4±0.4, and 12.3±0.80 g/dL (Table 2).

In our population, when pregnancy was diagnosed, 16.64% (n=870) were considered as having mild anemia, 3.07% (n=161) had moderate anemia, and 0.28% (n=15) had severe anemia (Table 2). The overall anemia prevalence at the time of pregnancy diagnosis was 20.0% (n=1046).

When the Hb levels were considered according to the age interval of the women, anemia prevalence was close to 20%

Table 1. Age and hemoglobin parameters of the participants

	n	Minimum	Maximum	Mean	Standard deviation
Hemoglobin	5228	7.07	15.56	11.88	1.15
Age	5228	17	46	30.21	4.75

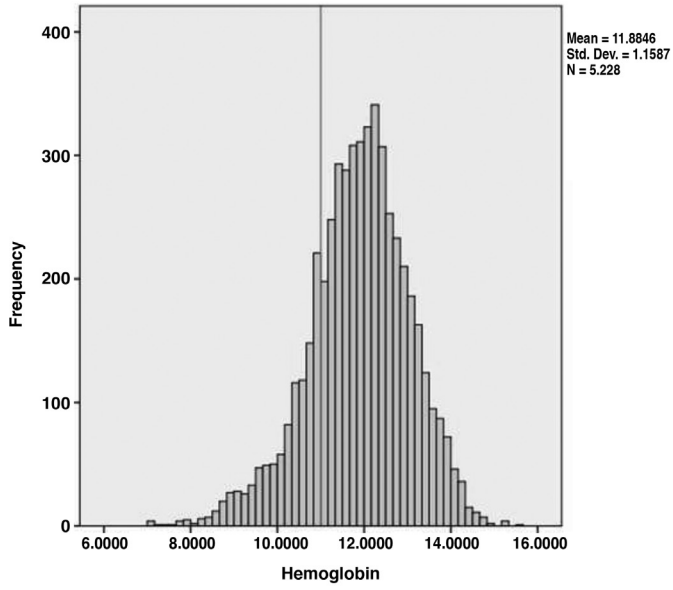

Figure 1. Distrubition of hemoglobin parameters of the pregnant women

in those aged 25-34 years (Table 3). Of the pregnant women, 7.3%, 21%, 20.5%, 19.9%, and 13.9% had anemia in the age groups 17-19 years, 20-24 years, 25-29 years, 30-34 years, and over 40 years, respectively (Table 3) (Figure 2).

Discussion

Anemia in pregnancy is a global public health problem. The prevalence is 18% in developed countries, whereas it is between 35-75% in developing countries[9]. In developing countries, it has been estimated that 460 million women of reproductive age are anaemic, 2/3 of whom are in Asia. It is known that prevalence of anemia in pregnancy is 42% worldwide, the lowest being 6% in North America and the highest is 75% in Gambia[3]. The prevalence of anemia in pregnancy is 25.1% in Europe, and around 24.1% in America[10]. In a study perfomed in China with 88149 pregnant women, the prevelance of anemia in the first trimester was determined as 22%[5].

In our country, in a study performed in 2006 in which 586 pregnant women were included, the prevalence of anemia was

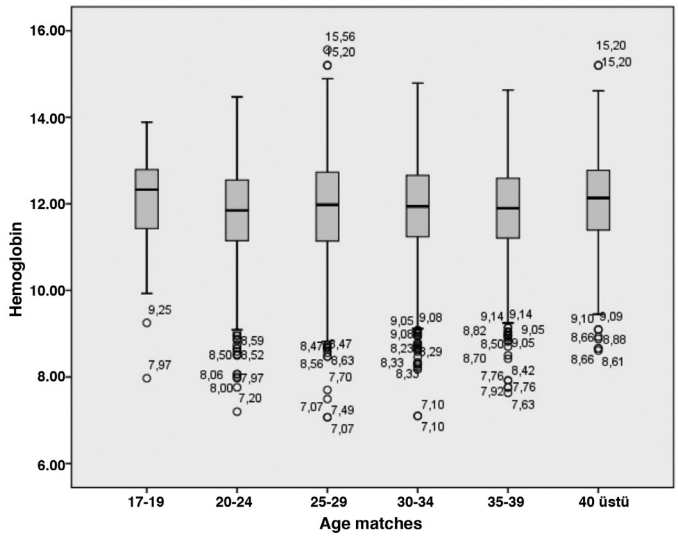

Figure 2. Distribution of pregnant women according to age and hemoglobin parameters

Table 2. Age and hemoglobin parameters of the participants according severity groups

Group		n	Minimum	Maximum	Mean	Standard deviation
Severe anemia (0.24%)	Hemoglobin	15	7.07	7.97	7.56	0.356
	Age	15	19	39	30.40	7.149
Modarate anemia (3.07%)	Hemoglobin	161	8.00	9.49	9.00	0.347
	Age	161	18	44	30.02	5.42
Mild anemia (16.64%)	Hemoglobin	870	9.50	10.99	10.46	0.410
	Age	870	18	46	30.14	4.465
Higher than hemoglobin 11 g/dL (80%)	Hemoglobin	4182	11.00	15.56	12.30	0.807
	Age	4182	17	46	30.24	4.780

Table 3. Distribution of pregnant women with anemia according to age ranges

Age (years)	n		Minimum	Maximum	Mean	Standard deviation
17-19	Hb <11 mg/dL (7.3%)	3	7.97	9.93	9.05	0.99
	Hb >11 mg/dL (82.7%)	38	11.09	13.89	12.31	0.75
20-24	Hb <11 mg/dL (21%)	116	7.20	10.99	9.99	0.85
	Hb >11 mg/dL (79%)	433	11.00	14.47	12.21	0.74
25-29	Hb <11 mg/dL (20.5%)	372	7.07	10.99	10.21	0.69
	Hb >11 mg/dL (79.5%)	1434	11.00	15.56	12.34	0.83
30-34	Hb <11 mg/dL (19.9%)	364	7.10	10.99	10.27	0.69
	Hb >11 mg/dL (80.1%)	1463	11.00	14.79	12.30	0.79
35-39	Hb <11 mg/dL (19.9%)	172	7.63	10.99	10.18	0.75
	Hb >11 mg/dL (80.1%)	697	11.00	14.63	12.26	0.80
Over 40	Hb <11 mg/dL (13.9%)	19	8.61	10.97	9.83	0.85
	Hb >11 mg/dL (86.1%)	117	11.03	15.20	12.39	0.87

Hb: Hemoglobin

determined as 74.1%[2]. In that study, the threshold Hb level for anemia was taken as 11 g/dL[2]. This level is close to that of underdeveloped countries. The prevalance of anemia in pregnancy in Turkey was determined as 40.2% by the WHO according to data observed before 2000[3]. In some Turkish studies, the prevalance of anemia during pregnancy was identified as 29.4% in Afyon, whereas it was 42.4% in Elazığ[11,12]. In a study by Karaoglu et al.[13] with 823 pregnants, the prevalence of anemia was detected as 27.1%. The study was performed in Malatya and the Hb level was accepted as 11 g/dL[13].

In the present study, the prevalence of anemia in pregnancy was determined as 20% in 5228 pregnant women in Ankara. In the study performed by Karaoglu et al.[13], it was found that 0.48% of the pregnant women had severe anemia (under 8 g/dL); this rate was 0.28% in our study. This may be due to differences between patient populations in Ankara and Malatya.

When anemia prevalence was considered according to age intervals, Karaoglu et al.[13] found that the rate was about 30% in pregnant women aged 30-39 years, wheras it was around 20% in our study for women aged 20-39 years. In a study by Pirinççi et al.[12] that included data before 2001 in 465 pregnant women in Elazığ, it was shown that 42.4% (n=197) of patients had anemia (Hb levels below 11 g/dL); 44.8% of these were observed in the first trimester. Beside this, the authors reported the incidence of anemia as 59.4% for patients aged 19 years and below, 40.8% in the 20-29 years age group, 39.5% in the 30-39 years age group, and 25.0% in the >40 years age group[12].

In our 2011-2015 data, the anemia prevalance was detected as 7.3% in patients aged 19 years and below, 26.1% in the 20-29 years age group, 24.8% in the 30-39 years age group, and 13.9% in the >40 years age group. In our study, the prevalence of anemia "at the time of pregnancy diagnosis" was determined as 20.1% because fertility is higher between the ages of 20-39 years. When our results are compared with those of Pirinççi et al.[12], one might conclude that the prevalence of anemia decreased by half during this 10-year period. The effect of socioeconomic differences between Elazığ and Ankara and the presence of a more conscious pregnant population could also account for this difference. It was revealed that the incidence of anemia during pregnancy increased as pregnancy advanced (1.8% in the first trimester, 8% in the second trimester, and 27% in the third trimester)[14].

In the studies mentioned above, the measurements for determining the prevalance of anemia were performed with regard to the number of weeks' pregnancy. However, in our study, the prevalance of anemia was investigated in pregnant women in the first trimester only, and this could account for the differences between the rates observed in the indicated studies and our study.

Anemia in the first trimester of pregnancy increases the risk of preterm birth, small-for-gestational-age births, and intrauterine growth restriction[15]. For mothers, severe anemia is an important risk factor for morbidity and mortality in developing countries. The risk of operative birth and prolonged delivery increases in cases of severe anemia[15].

Study Limitations

This study has a limitation, the anemia prevalence in pregnancy "at the time of pregnancy diagnosis" was detected as 20% and this was close to the rates of developed countries. However, this rate could be related to the patient population of Ankara where the socioeconomic level is higher in comparison with other regions of our country.

Conclusion

Anemia in pregnancy is a global public health problem and early diagnosis and treatment are both maternally and perinatally important. Detecting patients with anemia in the preconceptional period and/or delaying pregnancy until optimal Hb levels are reached will lower this rate.

Ethics

Ethics Committee Approval: The study was approved by the Etimesgut Military Hospital Local Ethics Committee (approval number: 8000-11-12).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.96641

Turk J Obstet Gynecol 2017;14:181-6

A new operation technique for uterine prolapse: Vaginally-assisted laparoscopic sacrohysteropexy

Uterin prolaps için yeni bir operasyon tekniği: Vajinal asiste laparoskopik sakrohisteropeksi

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Abstract

Objective: To describe the new surgical technique and report the safety and feasibility of vaginally-assisted laparoscopic sacrohysteropexy (VALSH).
Materials and Methods: Thirty-three women with stage 3 or more uterine prolapse underwent VALSH operation. Patients were followed up for 12 months for mesh-related complications and improvements of symptoms. The operation had three sections; 1st laparoscopic, 2nd vaginal, 3rd laparoscopic.
Results: The mean age, gravidity, and parity of the study population were 46.5 years (range, 25-68 years), 4.3 (1-9), and 2.9 (1-6), respectively. The mean duration of operation was 59.5 min (range, 20-120 min). There were significant differences between the pre- and post-operative values of pelvic organ prolapse quantification parameters, which were favorable in the latter evaluation ($p<0.001$); total vaginal length was preserved after surgery ($p>0.05$).
Conclusion: VALSH is a safe and minimally-invasive procedure in uterovaginal prolapse, with favorable anatomic and functional outcomes at 12 months post-operatively.
Keywords: Sacrohysteropexy, laparoscopy, vaginally-assisted laparoscopic sacrohysteropexy

Öz

Amaç: Yeni bir cerrahi yöntem tanımlamak ve vajinal asiste laparoskopik sakrohisteropeksi (VALSH) operasyonunun güvenilirliği ve yapılabilirliğini rapor etmektir.
Gereç ve Yöntemler: Evre 3 veya daha ileri düzeyde uterin prolapsusu olan 33 hasta VALSH operasyonuna alındı. Hastalar meşe bağlı komplikasyonları ve semptomları değerlendirme amaçlı 12 ay takip edildi. Operasyonun üç bölümü vardı: 1. laparoskopik, 2. vajinal, 3. laparoskopik.
Bulgular: Çalışma grubunda ortalama yaş, gravidite, ve parite sırası ile 46,5 yıl (25-68 yıl), 4,3 (1-9), ve 2,9 (1-6). Ortalama operasyon süresi 59,5 dk (aralık, 20-120 dk). Sonraki değerlendirmede daha iyi olmak üzere, operasyondan önceki ve sonraki pelvik organ prolapsus-sınıflaması değerlerinde anlamlı fark izlendi ($p<0,001$); total vajinal uzunluk cerrahi sonrası korundu ($p>0,05$).
Sonuç: VALSH uterovajinal prolaps tedavisinde güvenli ve minimal invaziv, anatomik ve fonksiyonel sonuçları iyi olan bir prosedürdür.
Anahtar Kelimeler: Sakrohisteropeksi, laparoskopi, vajinal asiste laparoskopik sakrohisteropeksi

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Received/Geliş Tarihi: 22.05.2017 **Accepted/Kabul Tarihi:** 30.08.2017

PRECIS: Vaginally-assisted laparoscopic sacrohysteropexy is a safe, minimally-invasive procedure in uterovaginal prolapse. Twelve months postoperatively, this procedure showed favorable anatomic and functional results.

Introduction

Uterovaginal prolapse is a frequently encountered condition especially among older women, which may lead to disability and poor quality of life[1], and it is a major indication for gynecologic surgery. The lifetime risk for a woman to have uterine prolapse surgery is 11%[1]. Risk of developing this kind of disorder increases with advancing age[2]. Surgery should be performed to restore anatomy with minimal morbidity and the lowest risk of recurrence. Three different approaches have been introduced to repair the pelvic floor such as abdominal, vaginal, and laparoscopic techniques. Hysterectomy is still considered to be the standard procedure for correcting uterovaginal prolapse[3]; however, in the majority of cases, hysterectomy does not overcome abnormalities associated with weakened pelvic support structures such as uterosacral and cardinal ligaments[4]. Additionally, due to the belief that the uterus plays a role in sexual satisfaction, an increasing number of women avoid undergoing hysterectomy[5]. Hysterectomy was shown to be associated with increased morbidity, blood loss, operative time, and influence post-operative recovery time[6]. Sacrospinous hysteropexy has been proposed to be an alternative approach for uterine-preserving prolapse surgery[7]. Other management alternatives include transvaginal mesh kits[8,9], abdominal sacrohysteropexy using mesh[10], and laparoscopic uterine suspension using a sling[11] or mesh[12]. A modified form of uterine-preserving prolapse surgery using a combined vaginal and laparoscopic approach was introduced and a series of 70 women was reported by Fayyad and Siozos[13].

In this case series, we describe the safety, feasibility, and outcomes of a modified form of a combined vaginal and laparoscopic approach, vaginally-assisted laparoscopic sacrohysteropexy (VALSH) for treating advanced uterovaginal prolapse.

Materials and Methods

This study is a descriptive prospective case series of 33 women with symptomatic stage 3 or 4 uterine prolapse on the pelvic organ prolapse-quantification (POP-Q) prolapse examination system[14] who underwent VALSH between 2012 and 2015 in Zeynep Kamil Women and Children's Health Training and Research Hospital. The procedure was approved by the Zeynep Kamil Women and Children's Health Training and Research Hospital Local Ethics Committee (approval number: 2015/195) and informed consent forms were obtained from each patient. This new alternative of surgical intervention was offered to women with advanced uterine prolapse.

Inclusion criteria were as follows:

(1) Symptomatic uterine prolapse > stage 3; (2) symptoms of pelvic organ prolapse such as vaginal bulge and heaviness.

Women were asked to empty their bladder before examination. All examinations were performed while the women were in the lithotomy position. The women were asked to perform a maximum Valsalva maneuver before genital examination. Stages of uterine prolapse were determined according to the POP-Q classification. Preoperatively, all patients underwent POP-Q questionnaires to assess prolapsed-related symptoms[15]. After determining the surgical indication and following preoperative preparations, the procedure was performed under general anesthesia with the patient initially in the lithotomy position. Thirty-three women with stage 3 or more uterine prolapse underwent VALSH operations. Patients were followed up over a 12-month period after surgery for mesh-related complications and improvements of symptoms.

The operation consisted of three sections; 1st laparoscopy, 2nd vaginal, 3rd laparoscopy. Laparoscopic ports were placed; one 10-mm port for umbilical and two or three 5-mm ports for lateral or suprapubic sites based upon conditions in order to achieve optimal surgical site exposure. The peritoneum over the sacral promontory was incised. A small 5-cm tunnel was made underneath the peritoneum from the sacral promontory downward to the cervix. Then, a lightweight type of mesh was placed onto the promontorium surface.

Second, the vaginal part of the surgery was performed. A semicircular incision was made at the posterior cervicovaginal junction. Curved ring forceps were placed into the retroperitoneal area via a tunnel established through blunt dissection from the vaginal part towards the promontory. At the same time, the direction of the instrument was visualized via laparoscopy (Figure 1). When the tip of the ring forceps was observed through the incision in the promontorium, the mesh was grasped and pulled downward within the tunnel with the aid of an instrument. The mesh was then fixed onto the posterior face of the uterine cervix using 3-4 non-absorbable

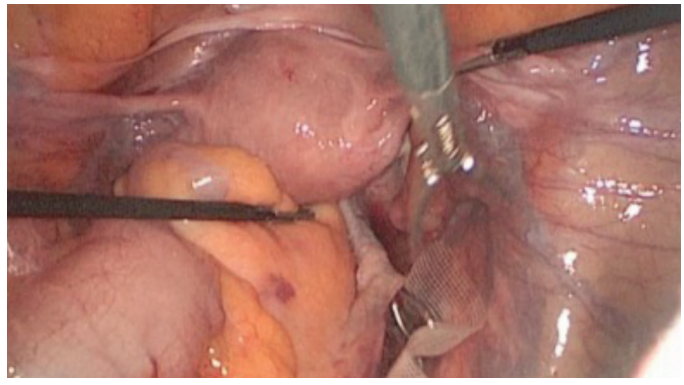

Figure 1. Laparoscopic view of curved ring forceps put into the retroperitoneal space via incision performed on the posterior wall of cervix

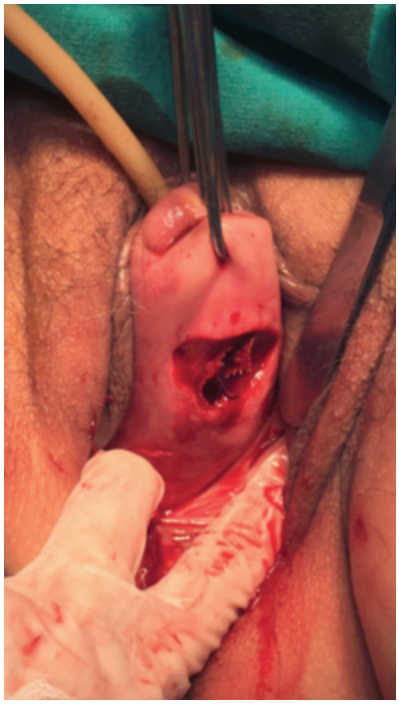

Figure 2. Cervical incision performed on the posterior wall where curved ring forceps are introduced and later mesh is fixed by 2-0 non-absorbable sutures x4

sutures via the vaginal route (Figure 2). The vaginal incision was closed by absorbable sutures.

Finally, the uterus was pushed up to the maximum level using a Rubin's cannula to obtain the required uterine suspension and mesh was tacked/sutured to the anterior longitudinal ligament at the sacral promontory and the peritoneal membrane over the promontory was sutured via the laparoscopic approach. A transobturator tape insertion procedure was applied in patients with stress urinary incontinence diagnosed before the operation through appropriate urogynecologic tests. A post-operative examination was performed in the lithotomy position using the POP-Q system. Mesh complications including mesh exposure were noted.

Statistical Analysis

Data were analyzed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, Illinois). The pre- and post-operative data were compared using the Wilcoxon t-rank test, and p values of <0.05 were considered significant.

Results

The mean age, gravidity, and parity of the study population were 46.5 years (range, 25-68 years), 4.3 (1-9), and 2.9 (1-6), respectively (Table 1). The mean operation time was 59.5 min (range, 20-120 min). Pre- and post-operative symptoms are

Table 1. Summary of some demographic features of the study population

	n	Minimum	Maximum	Mean	Standard deviation
Age (years)	33	25.0	68.0	46.5	10.3
BMI (kg/m^2)	33	21.00	33.00	28.5	3.5
Gravidity	33	1.0	9.0	4.3	1.8
Parity	33	1.0	6.0	2.9	1.2

BMI: Body mass index

Table 2. Summary of pre- and post-operative urogynecologic symptoms of the whole study population

	Symptoms				
	Pre-operative		Post-operative		
	Frequency	Percent	Frequency	Percent	p
Difficult defecation	3	9.1	1	3	
Stress urinary incontinence	1	3.0	0	0	
Stress urinary incontinence, mass protruding from vagina	4	12.2	0	0	
Stress urinary incontinence, difficult defecation	1	3.0	1	3.0	
Difficult defecation and urination	2	6.1	0	0	<0.05
Mass protruding from vagina with Valsalva	15	45.4	0	0	
Stress urinary incontinence, difficult urination	4	12.1	2	6.1	
Frequency, stress urinary incontinence	2	6.1	1	3	
Constipation, mass protruding from vagina	1	3.0	0	0	
Total	33	100.0	33	100.0	

shown in Table 2. The comparison of pre- and post-operative hemoglobin levels with the parameters of POP-Q values are summarized in Table 3. The rates of pre-operative prolapse stages were 4Ba (n=12, 36.3%), stage 4C (n=10, 30.3%), stage 3 Ba (n=3, 9.1%), stage 3C (n=3, 9.1%), stage 4 Bp (n=2, 6.1%), stage 4D (n=2, 6.1%), and stage 3 Bp (n=1, 3%). There were significant differences between the pre- and post-operative values of POP-Q parameters, which were favorable in the latter evaluation ($p<0.001$); total vaginal length was preserved after surgery ($p>0.05$). Among 33 women, stress urinary incontinence was determined in 12 patients by prolapse reduction testing (36.4%) managed by combined VALSH and transobturator tape insertion procedures. No perioperative complications were observed. There were five patients with cervical elongation concomitant with the uterine prolapse. At 12 months after surgery, all 33 patients reported cure of their prolapse symptoms with a subjective cure rate of 100%. None of the patients developed de novo urgency, infection or mesh erosion following surgery during the follow-up period. On assessing the patients' global impression of improvement, all 33 women reported feeling either "very much better" or "much better." No recurrence or mesh complication was observed after 12 months' follow-up.

Discussion

In this case series, we wanted to show the feasibility of a new technique of VALSH. Data from our series show this technique to be feasible, safe, and easy to perform. It has several advantages over conventional techniques and recently introduced new vaginal-assisted laparoscopic approaches. There is still no consensus on the optimal management of advanced uterine prolapse. Vaginal hysterectomy has been proposed to be a standard procedure in these cases. In order to avoid removing a healthy organ and increasing morbidity and mortality with the hysterectomy procedure and also preserve fertility, uterine-preserving approaches were introduced a few decades ago[10-12]. With the recent advances in endoscopic surgery, some laparoscopic uterine suspension techniques have been described[16-18]. According to the accumulated data, independent from the removal of the uterus, apical suspension is the required step for successful outcomes[19]. Recent studies showed some advantages of sacrohysteropexy including low recurrence rates, absence of mesh erosion, preserving an adequate vaginal length, and maintaining the proper physiological vaginal axis[3]. Additionally, laparoscopic hysteropexy was shown to be associated with better anatomic cure rates of higher than 90% in the majority of the studies. There was an improvement in symptoms, and cure rates of 73-100% among the patients. Reoperation rates and complication rates were generally low[20]. Furthermore, endoscopic approaches have some well-defined advantages including quicker recovery and a reduction in adhesion formation[12,13]. Additionally, current laparoscopic techniques provide better and magnified visualization of the anatomy and better hemostasis resulting from intraperitoneal gas pressure[13]. In the literature, one of the techniques of laparoscopic ventrosuspension of uterus used the rectus sheath for this purpose. However, according to the reports from the literature, it had unsatisfactory results[21]. On the other hand, uterosacral plication and suture hysteropexy were reported to have 80% success rates[19,22]. Recently, total laparoscopic hysteropexy by mesh placement from the upper part of the cervix to the sacral promontory was developed[12]. In addition, a published cohort study on the long-term outcomes of laparoscopic versus vaginal mesh hysteropexy revealed high satisfaction rates for both procedures[23]. Most hysteropexy techniques have been shown to have high satisfaction and low reoperation rates. It was reported that the type of hysteropexy and possible graft configuration may impact reoperation rates for recurrent prolapse. Furthermore, authors claimed that vaginal mesh risks and the possibility of future hysterectomy with mesh-associated risks should also be considered[24]. Moreover, relative to conventional laparoscopic sacral hysteropexy, total laparoscopic hysterectomy with laparoscopic sacrocolpopexy

Table 3. Comparison summary of pre- and post-operative pelvic organ prolapse-quantification classification parameters

	Pre-operative	SD	Post-operative	SD	p
Hgb (gr/dL)	12.2	1.3	11.8	1.1	>0.05
Aa (cm)	2.06	0.6	-2.3	0.4	<0.001
Ba (cm)	3.6	1.8	-2.2	1.6	<0.001
C (cm)	3.2	2.8	-6.8	0.9	<0.001
gh (cm)	3.9	0.6	1.9	0.1	<0.001
pb (cm)	1.6	0.7	2.8	0.8	<0.001
tvl (cm)	7.3	0.7	7.4	2.9	>0.05
Ap (cm)	1.6	1.2	-2.1	0.5	<0.001
Bp (cm)	2.4	1.7	-2.3	0.7	<0.001
D (cm)	1.5	2.1	-7.3	1	<0.001

SD: Standard deviation

procedures were shown to have similar anatomic results, excellent patient satisfaction, and improved quality of life scores[25]. Therefore, some modified forms of laparoscopic hysteropexy operations have been introduced.

In their series, Fayyad and Siozos[13] aimed to report the results of a novel technique of hysteropexy using vaginal dissection and mesh placement and fixation of mesh to the sacral promontory via a laparoscopic view. It had some advantages as well as efficacy, especially in patients with cervical elongation and the extra-peritoneal attachment of the mesh to the cervix eliminated the risk of compromise of uterine blood flow. On the other hand, the theoretical disadvantage of this technique is that the insertion of the mesh vaginally can result in increased infection and mesh exposure rates[13].

In their study, Fayyad and Siozos[13] showed that their technique called "VALUES" was free from increased risk of vaginal shortening and narrowing. In addition, the procedure was shown to result in shorter hospital stay and quicker recovery[13]. Sacral colpopexy was reported to have a lower risk of recurrent prolapse on examination, redo surgery for prolapse, post-operative stress urinary incontinence, and dyspareunia than a variety of vaginal interventions. However, there is limited evidence to support the use of transvaginal mesh for apical vaginal prolapse repair. There is no consensus on the best access routes for sacral colpopexy. Moreover, there is no clear conclusion on the comparison between uterine-preserving surgery and vaginal hysterectomy for uterine prolapse[26]. Recent studies indicated the necessity of randomized controlled studies to show the benefits of laparoscopic sacrocervicopexy with or without supracervical hysterectomy in terms of surgical outcomes and reduced risk of mesh erosion compared with sacrocolpopexy and concomitant total hysterectomy[25,27]. Pelvic organ prolapses and stress urinary incontinence were reported to coexist in 80% of patients with pelvic floor dysfunction[28]. We detected coexistence of pelvic organ prolapses and stress urinary incontinence in 36.4% of cases, which was managed through transobturator tape insertion.

The most difficult part of the procedure during conventional sacrohysteropexy is to dissect the peritoneum down to the cervix. Hemorrhage during this dissection further deteriorates tissue exposure. Furthermore, this partially blinded dissection increases the risk of hypogastric nerve plexus injury. The cervical region in which the mesh is inserted is very close to the rectum, which further makes the procedure difficult. Also, it is difficult to fix the mesh low enough to the cervix in patients with cervical elongation, which results in unsatisfactory results.

Study Limitations

This study has some limitations, one of them is small sample size and study needs longer follow-up duration.

Conclusion

VALSH is a safe, minimally-invasive procedure in uterovaginal prolapse that preserves the uterus, enables future normal vaginal delivery, and has shown favorable anatomic and functional outcomes at 12 months follow-up including zero recurrence rates.

Ethics

Ethics Committee Approval: The study was approved by the Zeynep Kamil Women and Children's Health Training and Research Hospital Local Ethics Committee (approval number: 2015/195).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.S., M.P., Concept: İ.S., Design: İ.S., Data Collection or Processing: İ.S., Ç.K., M.P., E.Ö., M.D., A.K., Analysis or Interpretation: E.Ö., İ.S., Literature Search: E.Ö., İ.S., Writing: E.Ö., İ.S., M.D., S.G.K.

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

Financial Disclosure: The authors declared that this study received no financial support.

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Case Report / Olgu Sunumu

DOI: 10.4274/tjod.57984

Turk J Obstet Gynecol 2017;14:187-90

Isolated tubal torsion: Successful preoperative diagnosis of five cases using ultrasound and management with laparoscopy

İzole tubal torsiyon: Beş olgunun ultrasonografi ile başarılı preoperatif tanısı ve laparoskopi ile yönetimi

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Abstract

Our aim was to evaluate the presentation and diagnostic evaluation of patients with isolated tubal torsion and to evaluate the surgical approach to these patients. We also aimed to define the ultrasonographic diagnostic criteria. Five patients with isolated tubal torsion who were admitted to our gynecology department between January 2014 and January 2017 were evaluated and included in this study. All cases were diagnosed through ultrasonographic imaging alone. The preoperative findings of the patients were similar to those described in the literature. No further imaging modality was used for diagnosis and all patients were managed with laparoscopy. The clinical findings and ultrasonographic findings were consistent with literature. It may be difficult to preoperatively diagnose isolated tubal torsion, which is a rare clinical entity. Evaluation of these patients by an experienced sonographer and knowledge of the ultrasonographic findings of isolated tubal torsion may have vital preventive measures.

Keywords: Isolated tubal torsion, ultrasonography, whirlpool sign, case series

Öz

İzole tubal torsiyon tanısı alan hastaların klinik prezentasyonunu, preoperatif değerlendirmesini ve bu hastalara olan cerrahi yaklaşımı değerlendirmeyi amaçladık. Ayrıca bu hastaların tanısında kullanılan ultrasonografik tanısal kriterleri değerlendirmeyi amaçladık. Ocak 2014-Ocak 2017 tarihleri arasında hastanemiz jinekoloji kliniğine başvuran ve izole tubal torsiyon tanısı alan beş hasta çalışmamız kapsamında değerlendirilmiştir. Preopatif olarak yalnızca ultrasonografi kullanarak tanı almıştır. Hastaların preoperatif bulguları literatürde tarif edilen bulgularla benzerlik göstermektedir. Bu hastaların değerlendirilmesinde başka bir görüntüleme yöntemine ihtiyaç duyulmamış ve tüm hastalar laparoskopi ile yönetilmiştir. Klinik bulgular ve ultrasonografik bulgular ise literatürde sunulan bulgularla tutarlıdır ve ultrasonografik bulgular detaylı olarak sunulmuştur. Nadir bir klinik tablo olan izole tubal torsiyonun preoperatif doğru tanı alması zor olabilir. Bu hastaların tecrübeli bir sonografist tarafından değerlendirilmesi ve izole tubal torsiyona ait ultrasonografik bulguların bilinmesi hayati öneme sahip olabilmektedir.

Anahtar Kelimeler: İzole tubal torsiyon, ultrasonografi, burgaç bulgusu, olgu serisi

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Received/Geliş Tarihi: 08.04.2017 **Accepted/Kabul Tarihi:** 13.06.2017

Introduction

Isolated tubal torsion is a very rare entity, reported as 1 in 1.5 million women[1]. It is a challenging preoperative differential diagnosis because of its non-specific clinical findings such as lower abdominal pain, nausea, vomiting and fever, and should be kept in mind while approaching patients with abdominal pain[2]. This report presents five cases of isolated tubal torsion and their successful preoperative diagnosis using ultrasonography (USG) imaging and management with a laparoscopic approach at a tertiary health center within 2 years, and an evaluation of literature findings.

Case Report

Patients diagnosed as having isolated tubal torsion between January 2014 and January 2017 were included in this presentation after acquiring informed consent from all patients. Five patients with lower abdominal pain at different intensities were diagnosed as having tubal torsion using USG imaging. All five patients had significant acute lower abdominal pain. The patients described a sudden onset of symptoms, and four patients had slightly increased white blood cell counts (WBC). Only case 5 had a normal WBC count. Three of five patients had nausea and vomiting as other symptoms. The decision was made for all patients to undergo surgery with a preoperative diagnosis of tubal torsion with or without coexisting adnexal masses. No other imagining modality beyond USG was used for the preoperative diagnosis. All diagnoses were confirmed under laparoscopy, but coexisting risk factors were misdiagnosed in case 1 and 2, as listed at Table 1. Four of the five patients were managed with salpingectomy, and detorsion was performed in only one patient who desired future fertility. None of the patients had postoperative complications and all were discharged within 48 hours. The important feature of these five patients was the correct preoperative diagnosis acquired using just USG, which was confirmed during the operation, and also through postoperative pathologic examination of specimens.

Discussion

The potential risk factors for isolated tubal torsion are tubal pathologies such as hydrosalpinx, paratubal cysts or ovarian masses, and altered tubal function. However, normal tubal appearance was mostly found in cases of isolated tubal torsion[3]. One of our five cases had normal tubal structure, two had paratubal cysts, and one had hydrosalpinx, consistent with the literature.

The USG features of tubal torsion may vary widely. Preoperative suspicion may rise with an image of elongated, convoluted cystic mass, tapering as it nears the uterine cornua[4]. An increased

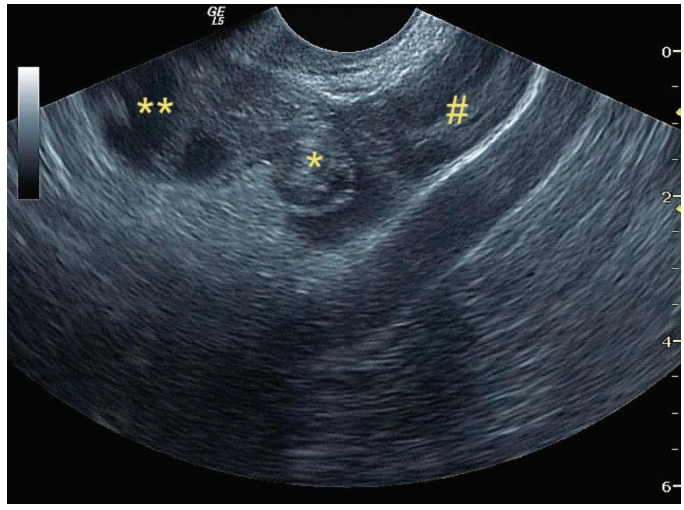

Figure 1. *"Whirlpool" sign, **hydrosalpinx at the distal side of tubal torsion, #ovarian tissue

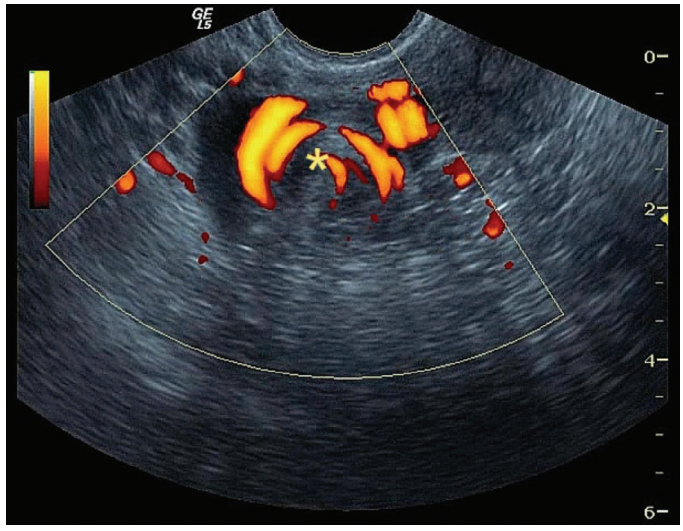

Figure 2. Ongoing circulation within the "whirlpool" sign showing circular pattern

Table 1. Summary of five cases with preoperative diagnosis and postoperative pathologic results

	Age (years)	Preoperative diagnosis	Surgery	Pathology result
Case 1	38	Tubal torsion + hydrosalpinx	Laparoscopic detorsion + paratubal cyst excision	Benign serous cyst
Case 2	49	Tubal torsion + paratubal cyst	Laparoscopic salpingectomy	Hydrosalpinx
Case 3	30	Tubal torsion	Laparoscopic salpingectomy	Dilated fallopian tube
Case 4	32	Tubal torsion + paratubal cyst	Laparoscopic salpingectomy	Paratubal benign cyst
Case 5	28	Tubal torsion + paratubal cyst	Laparoscopic salpingectomy	Hematosalpinx + paratubal benign cyst

resistance index due to decreased blood flow determined using Doppler USG may also strengthen the suspicion of torsion[5]. Nevertheless, abnormal Doppler findings are not a necessity for the diagnosis of torsion. Identifying a normal ipsilateral ovary may strongly suggest tubal torsion.

As in other torsion cases, a systematic evaluation of the adnexal areas is needed in order to be able to identify isolated tubal torsion cases with USG. A systematic evaluation of symptomatic patients should always include evaluation of tubal segments. In the correct evaluation of the adnexal area, the starting point should be the interstitial tubal segment, which is the starting point of the adnexa, and from here onwards, the entire tuba continues to medial to the fimbrial tip. Neighboring structures should also be evaluated at the same sections. The interstitial and trunk part of the uterine tube, the proper ovary ligament (ligamentum ovarii proprium) and the round ligament are located in the cornual area near the uterus. Over-torsions in this region are mostly around the proprium ligament axis, and tubal torsion mostly occurs by rotating around its own axis.

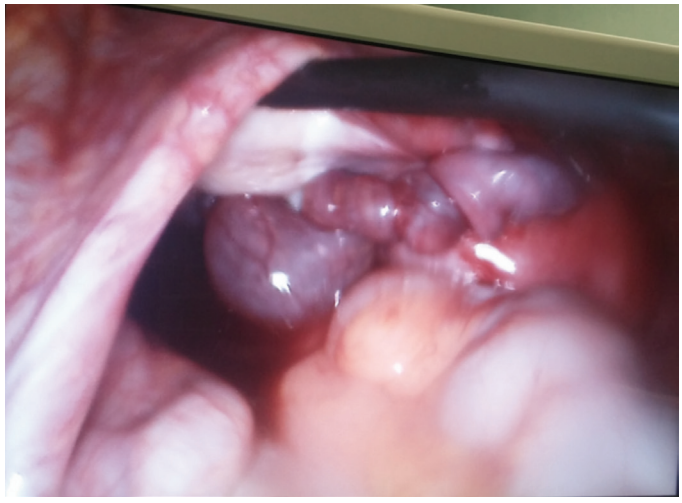

Figure 3. Isolated tubal torsion captured during laparoscopy without any other adnexal pathologies (case 3)

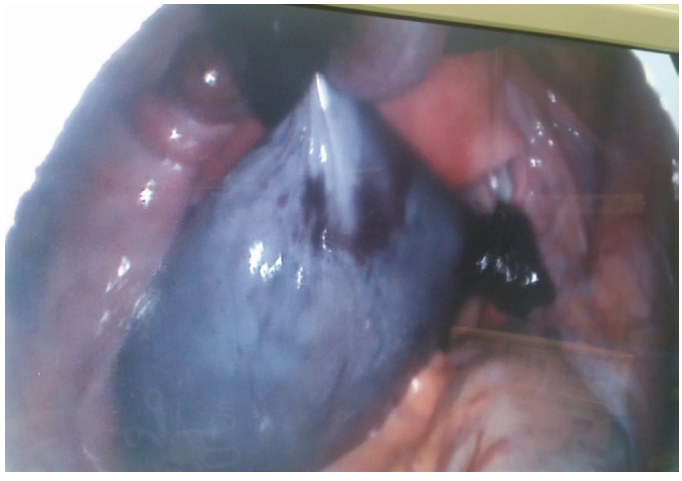

Figure 4. Isolated tubal torsion with concurrent paratubal cyst (case 5)

In isolated tubal torsion cases, torsional twist is directly observed on USG imaging (Figure 1). This finding must be seen and seen absolutely for the exact diagnosis of any kind of torsion in all cases. This finding is enough for the diagnosis of torsion because it is the physical image of torsion. Due to circulation problems, the tuba seems edematous and swollen and it is easily distinguishable from the overdose around the hypoechoic irregular soft tissue mass formed by the adnexal inferior tubal edema. When the "whirlpool" finding is evaluated with power Doppler, the venous circulation may be observed in a circular pattern (Figure 2). However, with the deterioration of the arterial circulation by the progress of the tubal torsion, this Doppler "whirlpool" image may disappear, but the sonographic image sign of turning around the axis remains. As the event progresses, the walls of the tuba become thicker with the edema and findings of hydrosalpinx and hematosalpinx in the lumen of the tuba begin to show themselves. Tubal torsion of this size may ultimately be regarded as ovarian torsion because the hematosalpinx is very enlarged and it is difficult to monitor the ovary when it starts to form incomplete septations.

The progression of torsion causes hematosalpinx, tubal rupture, and peritubal hematomas, which become more complicated and harder to diagnose. If the heterogeneous mass forming in the adnexal area is not carefully evaluated, it can easily be confused with a ruptured ectopic pregnancy.

The primary approach to tubal and ovarian torsion should be laparoscopy and recommended as primary approach[2]. Torsions are mostly treated with this approach owing to the advanced accessibility with laparoscopy. We prefer the laparoscopic approach for both ovarian and tubal torsions, as in these 4 cases, which were successfully managed using laparoscopy (Figure 3, 4).

In conclusion, tubal torsion is an emergency condition and a correct preoperative diagnosis should be acquired immediately. Ultrasound criteria for tubal torsion diagnosis require careful evaluation, thus it should only be performed by an experienced practitioner, and laparoscopy should be the primary choice of treatment.

Literature review

Isolated tubal torsion is a very rare clinical entity. Correct diagnosis requires great caution and experience because of it is rarity and non-specific symptoms. The most common symptoms were listed as abdominal pain, vomiting, and fever in pediatric case series[6]. Also, bowel and bladder problems, lower abdominal mass diagnosed during examination, and elevated WBCs may be other symptoms[7]. The etiology remains unclear, but anatomic changes, positional changes, trauma, previous surgeries or gravid uterus are listed as potential risk factors[8]. There is also a higher probability for right tubal torsion then left due to the position of the sigmoid colon and

slow venous drainage of the right tuba[9]. Paratubal or adherent cysts may also play an important role in the etiology according to literature and our findings[10]. This situation may be seen in pregnant patients; isolated tubal torsion should be considered as a diagnosis because of difficulties in imaging[11]. Magnetic resonance imaging (MRI) may be used with clinical suspicion both in pregnant and non-pregnant patients for differential diagnosis[9,12]. MRI may also show the "whirlpool" sign, which is the image of physical torsion of tubas[13].

Diagnosing torsion correctly using USG is also challenging, but very important because of its speed and easy accessibility. Ultrasonographic findings listed in the literature are mostly the same as the criteria used at our clinic. MRI or computed tomography scans may play role in diagnosis, but have disadvantages such as cost, radiation exposure, and potential hazards during pregnancy[14]. Prompt diagnosis is also very important due to the possible results of delay such as necrosis[15]. Management of tubal torsion mostly requires surgical intervention. Salpingectomy may be performed if there is no further desire for fertility, but the proper approach should be detorsion of tuba for preserving fertility[14]. Urgent intervention should be performed in symptomatic patients and rare results of chronic tubal torsion such as tubal autoamputation should be known[16].

Isolated tubal torsion is a very rare entity and may be misdiagnosed due to its non-specific symptoms. All physicians must know the diagnostic criteria for prompt diagnosis and a proper evaluations should always include the adnexal area and tubas. A systematic approach to the adnexal area as mentioned may diagnose most tubal torsion cases. After proper preoperative diagnosis, tubal torsions may be managed via laparoscopy. In conclusion, the most important point in managing these patients is a correct and rapid diagnosis.

Ethics

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.D., M.K., Concept: E.D., Ç.Ö., Design: R.D., Ö.L.T., Data Collection or Processing: E.F., E.D., Analysis or Interpretation: E.D., Ç.Ö., Literature Search: E.F., Ş.F., Writing: E.F., Ş.F.

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

Financial Disclosure: The authors declared that this study received no financial support.

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Case Report / Olgu Sunumu

DOI: 10.4274/tjod.34270

Turk J Obstet Gynecol 2017;14:191-4

Laparoscopic repair of ureter damaged during laparoscopic hysterectomy: Presentation of two cases

Laparoskopik histerektomide yaralanan üreterin laparoskopik tamiri: İki olgunun sunumu

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Abstract

Ureter injuries are uncommon but dreaded complications in gynecologic surgery and a frequent cause of conversion to laparotomy. Recently, a few papers reported the repair of gynecologic ureteral injuries using laparoscopy with encouraging results. In these case reports, we aimed to present two laparoscopically repaired ureter injuries during total laparoscopic hysterectomies (TLH). In the first case, the ureter was transected during the dissection of the cardinal ligament, approximately 7 to 8 cm distal to the ureterovesical junction (UVJ), and in the second case, it was damaged approximately 10 cm distal to the UVJ. Both transections were identified during surgery. The injured ureter was repaired without converting to laparotomy or additional trocar insertion. Ureteroureterostomy was performed in both cases uneventfully. Although ureteric injury is a rare complication during TLH, it can be managed by the same surgeon laparoscopically during the same procedure.

Keywords: Ureter, injury, complication, laparoscopy

Öz

Üreter hasarı, jinekolojik cerrahilerde nadir görülen ancak korkulan bir komplikasyondur ve tamiri için sıklıkla laparotomiye ihtiyaç duyulur. Yakın zamanda, jinekolojik üreter hasarının laparoskopik tamiri ile ilgili cesaretlendirici birkaç makale yayınlanmıştır. Amacımız total laparoskopik histerektomide (TLH) hasarlanan ve laparoskopik olarak başarılı bir şekilde tamir edilen iki olguyu sunmaktır. İlk olguda, sağ kardinal ligament (KL) hizasına kadar sorunsuz devam eden operasyonda, KL diseksiyonu sırasında üreterovezikal bileşkenin (UVB) 7-8 cm distalinde üreter hasarı oluştu. İkinci olguda, UVB'nin yaklaşık 10 cm distalinden üreter hasarlandı. Her iki üreter hasarı da cerrahi sırasında fark edildi. Hasarlanan üreterler laparotomiye dönmeden ve ek trokar girişi yapılmadan sorunsuz bir şekilde onarıldı. Her iki olguda da mid-üretral hasar oluştuğundan üreteroüreterostomi tercih edildi. Üreter hasarı her ne kadar TLH sırasında nadir gelişen bir komplikasyon olsa da, eğer mümkünse, laparoskopik sütür ve düğüm teknikleri konusunda deneyimli bir jinekolog veya ürolog tarafından, laparoskopik olarak tamir edilmelidir.

Anahtar Kelimeler: Üreter, yaralanma, komplikasyon, laparoskopi

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Received/Geliş Tarihi: 26.05.2017 **Accepted/Kabul Tarihi:** 12.09.2017

Introduction

Urologic complications during gynecologic surgery are uncommon but dreadful. The reported incidence of ureteral injury related to gynecologic surgery varies between 2.5% and 12.1%[1]. Pelvic ureters are retroperitoneal structures that run from the renal pelvis to the bladder that can be injured during pelvic surgery at any point along their distal course. However, the approach of hysterectomy plays a role in the variation of incidence in total laparoscopic hysterectomies (LHs) (TLHs) compared with the abdominal and vaginal approach; the reported odds ratio for urinary tract injuries was 2.41 and 3.69, respectively[2]. Although the risk of ureter injury significantly decreases with the increasing experience of surgeons, ureter injuries also occur in the hands of experienced gynecologists because the indication for TLH is expanding and the difficulty of the operation is increasing. The question is whether complications of laparoscopic procedures such as ureteral injury could be managed successfully and effectively without converting to laparotomy. We report successful immediate primary laparoscopic repair of two cases of ureter transection during TLH and discuss possible causes that lead to ureter injury and make recommendations for prevention.

Case Report

A patient aged 48 years underwent TLH and bilateral salpingo-oophorectomy (BSO) due to abnormal uterine bleeding that was unresponsive to medical therapy. She had no previous history of abdominal surgery or any kind of disease causing pelvic adhesions. On exploration, the uterus was of 8 weeks' pregnancy in size, the bilateral adnexa were normal, and no pelvic adhesions or distortion of anatomy was observed. A four-trocar technique (out of main trocar, two at the left side and one at the right side) and Hohl manipulator (manufactured by Karl Storz, Germany) was used. The TLH was uneventful until the area of the cardinal ligaments (CLs). The ureter was transected with the Ligasure™ (Valleylab, Boulder, CO, USA) during the dissection of the right CL, 7 to 8 cm distant from the ureterovesical junction (UVJ) (Figure 1a). The transection was identified during the dissection and immediate repair was performed peroperatively without converting to laparotomy. The proximal and distal ureteral segments were identified, then sutured at the 6 o'clock position for approximation (Figure 1b). A double J ureteral stent was taken to the abdomen through the 5-mm lateral trocar and inserted in the ureter (Figure 1c). After the stent application, 3/0 Vicryl sutures were placed at the 9, 12, and 3 o'clock positions (Figure 1d). A Foley catheter

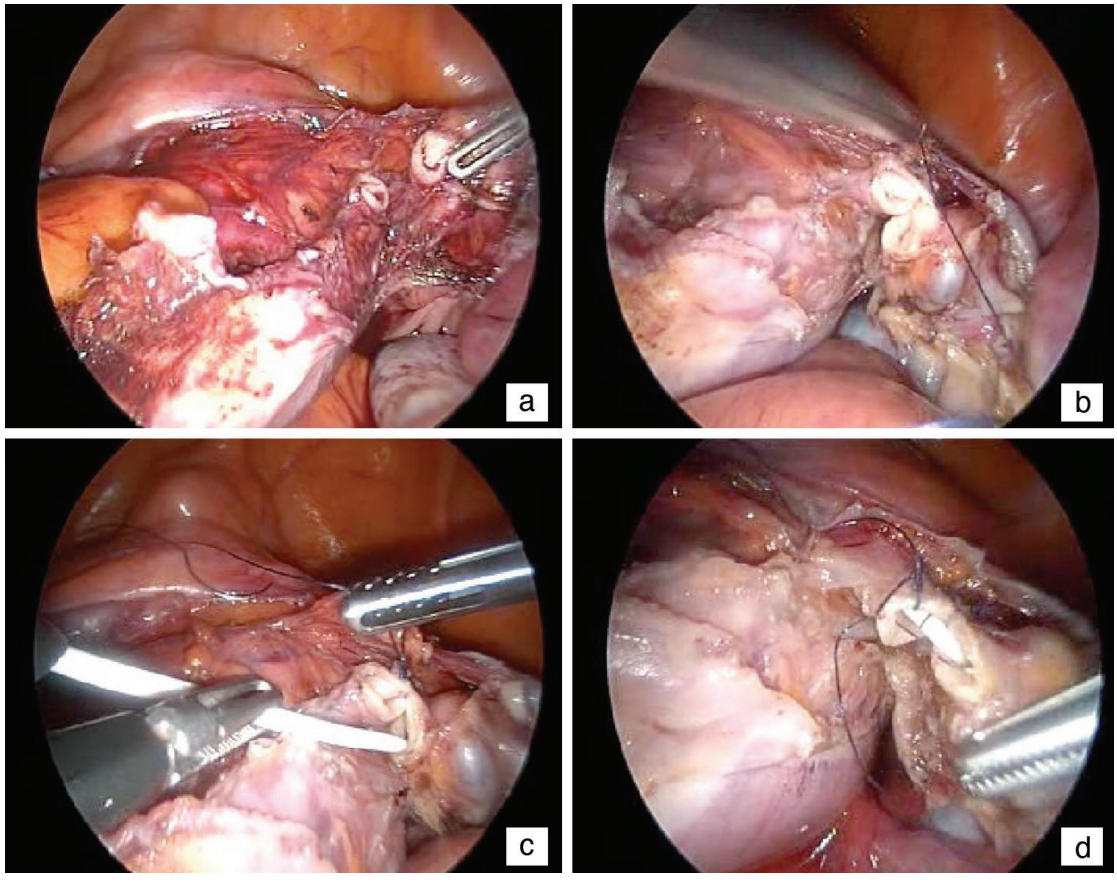

Figure 1. The surgical technique of laparoscopic ureter injury is shown. The transected edges of the ureter at the area of cardinal ligament are seen (a). First, the proximal and distal ureter edges are sutured at the 6 o'clock position (b), then the ureteral stent is inserted in the ureter (c) and the anastomosis is completed using sutures placed at the 9, 12 and 3 o'clock positions (d)

remained in the bladder and a drainage tube in the abdomen to prevent possible urinoma formation and to control bleeding. In the second case, a woman aged 59 years underwent TLH and BSO due to simple endometrial hyperplasia with atypia. TLH, BSO, and frozen pathology were planned with the suspicion of malignancy. Laparoscopy was performed using the same technique as in the first case. Frozen section was reported in favor of benign pathology. During the dissection of the left CL, the ureter was damaged approximately 10 cm distant from UVJ with the Halo™ bipolar cutting forceps (Gyrus ACMI, Olympus) (Figure 2). The injured ureter was noticed during the dissection and repaired without converting to laparotomy or additional trocar insertion, similar to the first case. The duration of the ureteral repairs from the time of the injury to the end of the last anastomosis suture were 55 and 40 minutes for the first and second cases, respectively. Estimated blood loss during the ureteral repairs was negligible. The vesical catheters were withdrawn on 7 days day postoperatively and the ureteral stents were removed cystoscopically on the 21st day. We did not use prophylactic antibiotics due to prolonged catheterization. Patients were closely monitored after discharge weekly for the first three weeks, then monthly up to 10-12 months using serial urinary system ultrasonography. Neither patient had any pelvicalyceal ectasia or visible ureters. The patients' recoveries were uneventful with normal kidney and urinary function. Written informed consent was obtained by both patients.

Discussion

Ureter injuries can result from pelvic dissection or due to thermal injury by excessive use of energy adjacent to the ureter. In a retrospective review of 165 patients with iatrogenic ureteral injuries over a 20-year span, endourologic procedures were responsible for most iatrogenic injuries with 42%, and gynecologic and general surgery procedures were 34% and 24%, respectively[3]. Urinary tract injuries during LH are reported to be more frequent than in abdominal or vaginal hysterectomies. The higher risk in LH compared with the abdominal approach is due to the use of electrocoagulation of uterine vessels during laparoscopic procedures[4]. Extensive electrocoagulation of uterine vessels and CLs near the ureter increases the risk of ureter injuries.

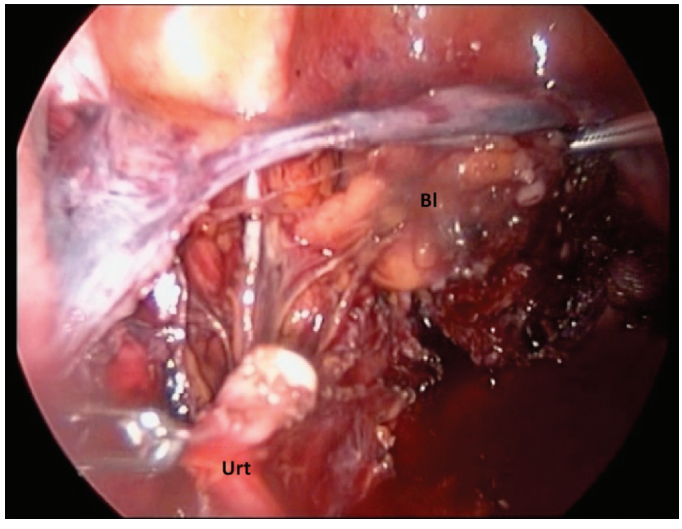

Figure 2. The transected edges of the ureter at the level of left cardinal ligament were seen

Urt: Ureter, Bl: Bladder

Intraoperative detection of ureter injuries has been reported in only 5-13% of cases[3,4]. Among the acute injuries of the urinary tract, ureter injuries are most difficult to recognize as there often may be few or with no symptoms[1]. Intraoperative iatrogenic ureteral injuries, if recognized during the procedure, should be repaired at that setting[5]. In our cases, the transected edges of the ureter were seen without any urine leakage during the dissection of the CLs.

Approximately 90% of the trauma to the ureter occurs in the lower portion, which extends from the inferior border of the sacroiliac joint to the UVJ[6]. Transection injuries are repaired depending on the severity of the injury and approximation to the UVJ. If the ureteral injury is more than 6 cm distant from the UVJ, a primary ureteral anastomosis is performed. Primary ureteroureterostomy is the optimal technique when the anastomosis can be performed without tension and if the initial approach was laparoscopic[7]. Laparoscopic ureteroureterostomy was preferred in our cases because the mid-ureter was transected approximately 8 cm and 10 cm distant from the UVJ, respectively.

Laparoscopic ureteral repair is technically feasible and an alternative to open repair for immediate, early, and delayed diagnosis of ureteral injury. In the literature, although long-term outcome of laparoscopic repair is limited to small series and case reports, the results are excellent if the diagnosis and repair is performed at the time of injury[8]. A delay in diagnosis worsens prognosis because of infection, hydronephrosis, abscess, and fistula formation. No postoperative complication occurred in our cases, the patients have continued their lives with normal kidney function and no urinary incontinence for 10 and 12 months follow-up, respectively.

Recommended techniques for reducing the risk of urinary tract injuries were agreed upon by experts in a Delphi consensus procedure: routine use of uterine manipulator, coagulation of uterine vessels close to the uterus with a perpendicular approach from the ipsilateral side, and visualization or dissection of the ureter in the case of distorted anatomy[9]. Intraoperative routine use of cystoscopy was proposed to assess the ureter flow as a part of all TLHs. Cystoscopy during TLH is well tolerated and can reassure surgeons of immediate urinary tract injuries; however, it is an additional time-consuming procedure that is not required in most patients. Cystoscopy during TLHs was postulated to be cost effective if the rate of ureter injury exceeded 2%[10].

In the case of ureteral injury during laparoscopic gynecologic surgery, either a gynecologist or urologist who is experienced in suturing and knot-tying techniques can successfully perform a primary laparoscopic repair of the ureter. Visualization of the course of ureters at the beginning or end of the operation may prevent complications of the urinary tract and possible delayed diagnosis of ureteral injuries.

Ethics

Informed Consent: Written informed consents were obtained from the patients for publication of these case reports and accompanying images.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: The authors declared that this study received no financial support.

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Case Report / Olgu Sunumu

DOI: 10.4274/tjod.75735

Turk J Obstet Gynecol 2017;14:195-8

Ovarian metastasis of Müllerian adenosarcoma of the cervix with sarcomatous overgrowth

Serviksin sarkomatöz gelişim gösteren Müllerian adenosarkomunda over metastazı

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Abstract

The aim of this study is to present a rare case of Müllerian adenosarcoma of the cervix with ovarian metastasis and sarcomatous overgrowth. A gravida 2, para 2 woman aged 32 years with vaginal bleeding was admitted to the gynecology department. A 3-4 cm polypoid mass protruding from the cervix was detected in a pelvic examination. Total abdominal hysterectomy and bilateral salpingoopherectomy was performed because of metastatic implants on the right ovary. The pathologic evaluation revealed Müllerian adenosarcoma of the cervix with sarcomatous overgrowth and ovarian metastasis. After surgery, the patient was planned to undergo chemo- and radiotherapy. This is the first cervical Müllerian adenosarcoma case mentioned in the literature with metastasis to the ovary in a young woman. There is no optimal management option for cervical adenosarcomas due to the rarity of this phenomenon. Nevertheless, even if the patient is young and imaging techniques do not elucidate metastatic disease, surgeons should evaluate the ovaries for the spread of tumor, especially if histology reveals sarcomatous overgrowth.

Keywords: Cervix, Müllerian adenosarcoma, ovarian metastasis

Öz

Bu çalışmanın amacı, nadir görülen ve over metastazı ile birlikte olan sarkomatöz gelişim gösteren serviks adenosarkom olgusunu sunmaktır. Otuz iki yaşında gravida 2, para 2 olan olgu, vajinal kanama yakınması ile jinekoloji bölümüne başvurmuştur. Pelvik bakıda, serviksten vajene protrüde olmuş 3-4 cm polipoid yapıda kitle izlenmiştir. Total abdominal histerektomi ve over üzerindeki metastatik odaklar nedeniyle de bilateral salpingo-ooferektomi uygulanmıştır. Patolojik değerlendirme, over metastazının eşlik ettiği serviksin sarkomatöz gelişim gösteren Müllerian adenosarkomu olarak raporlanmıştır. Operasyon sonrası hastaya kemoterapi ve radyoterapi planlanmıştır. Bu olgu, literatürde genç hastada overe metastaz yaptığı gösterilmiş ilk serviksin Müllerian adenosarkom olgusudur. Olgunun nadir görülmesinden dolayı, uygun tedavi yaklaşımı ile ilgili yeterli bilgi yoktur. Ancak olgu genç ve görüntüleme yöntemlerinin sonucunda overlerin normal dahi olduğu belirtilse, sarkomatöz gelişim gösterdiği bildirilen olgularda, cerrah over metastazı riskini göz önünde bulundurmalıdır.

Anahtar Kelimeler: Serviks, Müllerian adenosarkom, over metastazı

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Received/Geliş Tarihi: 17.04.2017 **Accepted/Kabul Tarihi:** 27.07.2017

Presented in: 25th European Congress of Pathology on 31 August-4 September 2013 in Lisbon, Portugal.

Introduction

Müllerian adenosarcoma (MA) is relatively a rare type of mixed epithelial and mesenchymal tumor of the uterus. It is usually composed of a benign epithelial component and a low-grade stromal-sarcomatous component. Occasionally, the epithelial component may be atypical and sarcomatous overgrowth may be seen in the stromal component[1-3]. MA mostly occurs in the uterus but also in extra-uterine sites, particularly in the ovary.

The tumors generally originate from the uterine corpus/endometrium, and less frequently from the uterine cervix. Adenosarcoma usually presents with abnormal vaginal bleeding. If the tumor originates from the cervix, the tumor is commonly seen as a polypoid tissue protruding from the external cervical ostium like a cervical polyp on gross evaluation[3,4]. Cervical adenosarcoma, especially with sarcomatous overgrowth, is extremely rare. Distant metastasis is also extremely rare because the epithelial component is usually benign and the stromal component is generally low-grade[1]. In this report, we present a MA originating from the endocervical canal with sarcomatous overgrowth including heterologous elements and ovarian metastasis in a young woman.

Case Report

A gravida 2, para 2 woman aged 32 years with vaginal bleeding was admitted to the gynecology department. She reported having abnormal vaginal bleeding for a couple of years and a benign endometrial biopsy in another gynecology center 6 months ago. The pelvic examination revealed a 3-4 cm polypoid mass protruding from the cervix to the vagina. The uterus and the ovaries were normal in size. Transvaginal sonography revealed an irregular-shaped polypoid structure lying from the internal cervical ostium level to the vagina whose thickness was 16 mm. Magnetic resonance imaging (MRI) revealed a lesion starting from the endocervical canal lying to the vagina and there were no pathologic lymph nodes. Her serum CA125 and CA19-9 levels were within normal limits. A biopsy from the polypoid lesion revealed MA with sarcomatous overgrowth.

During surgery, metastatic implants were observed over the right ovary and the left ovary was solid in appearance. The outer surface of the uterus was normal. Total abdominal hysterectomy and bilateral salpingoopherectomy was performed. Three polyps were seen in the endocervical canal, which measured between 0.6x0.5x0.3 and 2x0.8x0.5 cm. The cut surfaces of the polyps were solid and grey-white in appearance. Hemorrhage was seen because of previous biopsy. The tumor had invaded the outer half of the myometrium macroscopically (Figure 1a, 1b).

The final report confirmed that the tumor originated from the endocervical canal. It invaded the lower uterine segment and the myometrium, less than the outer half, and consisted of elongated spindle cells with a myxoid background and few benign glandular formations. The sarcomatous stroma made up 80% of the tumor. In these areas, paucicellular and hypercellular morphology with myxoid stroma were seen. Low-grade and high-grade stromal components were mixed. Mitotic activity was 4 per 10 high-power fields (HPFs) and 2 per 10 HPFs in paucicellular and hypercellular areas, respectively. Immunohistochemistry showed positive immunoreactivity with vimentin and desmin, and negative immunoreactivity to actin, s-100, and CD99. The glandular epithelium was primarily of endocervical type without atypia and mitotic activity. Large foci of benign cartilage were seen in the sarcomatous areas (Figure 1c, 1d, 1e). There was no endometrial involvement (Figure 1f). The tumor invaded the ovaries and there were no lymphovascular space invasion. The ovarian metastasis showed the same morphology (Figure 2). Intraoperative peritoneal lavage cytology was positive for malignant cells. The patient was discussed in the gynecologic oncology board and at the present time she has been taking a chemotherapy regimen of ifosfamide and doxorubicin. Then she is planned to have both pelvic and vault radiotherapy.

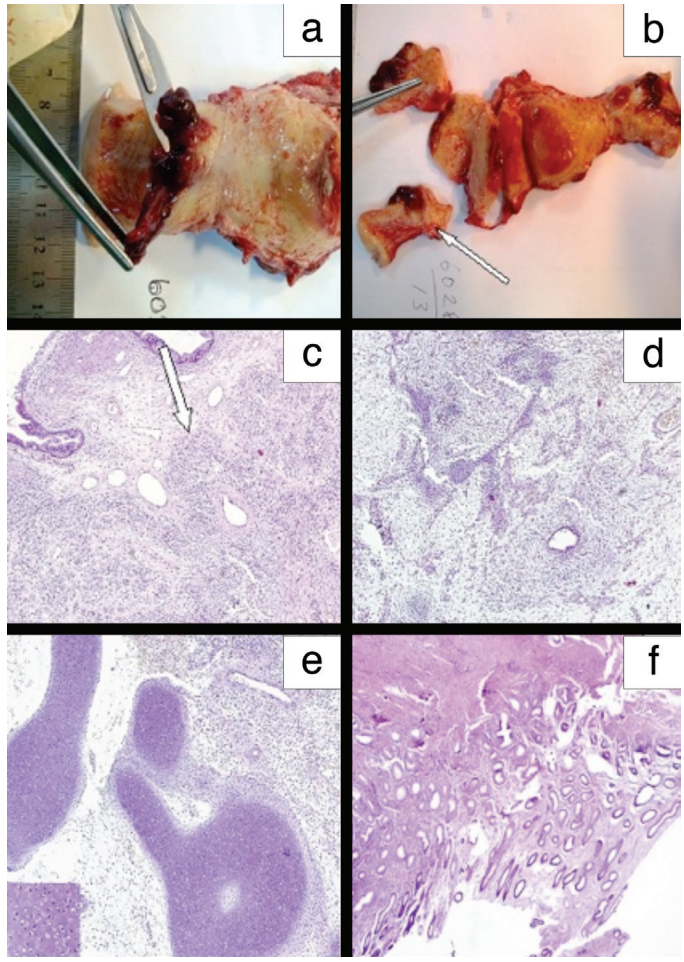

Figure 1. The tumor was seen as polyps (a) and invaded the outer third of the myometrium macroscopically (b). The tumor originated from the cervix, and sarcomatous component was over 25% (c, d). Large foci of benign cartilage (inset) were seen as heterologous element (e). The non-tumoral endometrium was seen (f)

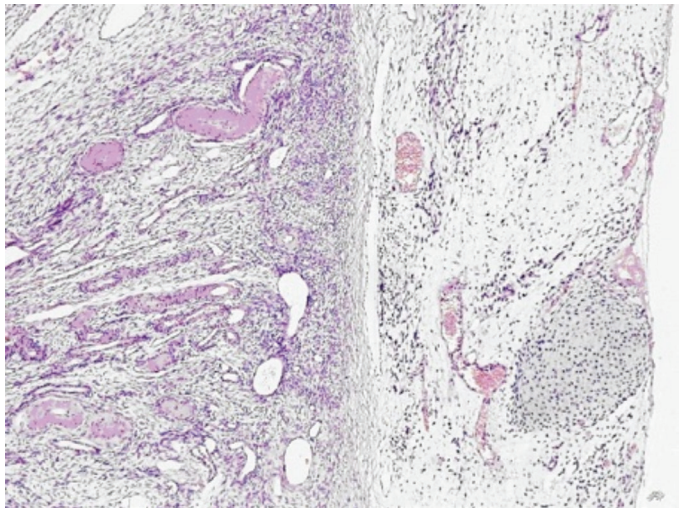

Figure 2. The ovarian metastasis showed sarcomatous component with myxoid background and foci of benign cartilage

Discussion

MA is an infrequent type of sarcoma of the female genital system. It mostly comprises a low-grade malignant stromal component and benign epithelial component. It commonly arises from the uterine corpus/endometrium. Cervical adenosarcoma is seen in 2-9% of all MAs[5-8]. The age at clinical presentation in cervical adenosarcoma is lower than that with uterine corpus, which is about 27 years[3-6]. This is concordant with our patient whose age was 32 years. Chin et al.[9] also showed that 89% of patients were premenopausal. The most frequent symptom is abnormal vaginal bleeding, as in our case[9]. Generally, the most common finding in physical examination is cervical polypoid lesion protruding from external cervical ostium to the vagina. The diagnosis can be made through biopsy from the lesion.

Sarcomatous overgrowth is defined as overgrowth of the neoplasm by a pure sarcomatous component occupying at least 25% of the lesion[1]. This subtype has a more malignant behavior than the classic adenosarcoma[10]. The rate of myometrial invasion and extension to the serosal surface, recurrence rate, and death because of tumor progression are all higher in adenosarcomas with sarcomatous overgrowth[10,11].

Heterologous elements are rarely reported, accounting for 8-42% of cervical adenosarcoma[3-4]. Mostly, these elements are cartilage or striated muscle. Charfi et al.[12] also reported a patient with adenosarcoma of the cervix with sarcomatous overgrowth and heterologous elements that contained cartilage and presented as a recurrent cervical polyp.

Cervical adenosarcomas with sarcomatous overgrowth in young patients are very rare. In particular, ovarian metastasis at the time of diagnosis is almost not mentioned in the literature. A recent study by Tanner et al.[13] reported that ovarian metastases were not identified in any patients undergoing bilateral salpingoopherectomy in 16 patients with MA. The present case is one of the first with cervical adenosarcoma metastases to the ovary.

The optimal therapy and the prognostic factors for these are still unclear because the reports of these cases are insufficient and long-term follow-up data are absent. The extensiveness of surgery may differ from patient to patient. Chin et al.[9], reported 9 cases of cervical adenosarcoma. They performed a cervical wedge resection for a patient who desired to preserve fertility. Hysterectomy, bilateral salpingoopherectomy, and lymphadenectomy were performed for other patients, none of whom had sarcomatous overgrowth. Park et al.[14] performed hysterectomy, bilateral salpingoopherectomy, and lymphadenectomy for a 37-year-old woman with adenosarcoma of the cervix with sarcomatous overgrowth. However, there was no tumor in the ovaries and no lymph node metastasis[14]. Manoharan et al.[15] reported three patients with cervical adenosarcoma. The first one was a 28-year-old woman with sarcomatous overgrowth. She underwent radical hysterectomy with preservation of the ovaries. The histology revealed lymphovascular space and right parametrial invasion, so she had chemotherapy followed by both pelvic radiotherapy and brachytherapy. The second case was a 26-year-old woman who had cervical MA with spindle cell stroma and benign glands. Vaginal hysterectomy with preservation of the ovaries and laparoscopic lymph node dissection was performed. The lymph nodes were free of tumor. She was given adjuvant pelvic radiotherapy because of deep stromal infiltration and high-grade tumor. The third case was a 41-year-old woman who had cervical adenosarcoma with mild-to-moderate stromal cytological atypia and underwent abdominal hysterectomy with preservation of ovaries. She received no adjuvant therapy because the tumor was localized to the polyp. Our patient was also a young woman and we were planning to preserve the ovaries before surgery. However, we came across the metastatic implants on the right ovary intraoperatively. MRI and ultrasonography did not facilitate the management preoperatively.

As previously mentioned, this is a very rare case and there is no consensus on either surgical or adjuvant treatment strategy. The first point that physicians should keep in mind is the number of mitotic figures in the mesenchymal part because the epithelial part is benign. Secondly, sarcomatous overgrowth may determine the behavior of the disease, as it was in our patient. If there is sarcomatous overgrowth, surgeons should carefully evaluate the patient for distant or locoregional spread. As a result, there is no optimal management option for cervical adenosarcomas due to the rarity of this phenomenon. Nevertheless, even if the patient is young and imaging techniques fail to reveal metastatic disease, surgeons should evaluate the ovaries for the spread of tumor, especially if the histology reveals sarcomatous overgrowth.

Ethics

Informed Consent: Consent form was filled out by the patient.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K., B.S., N.Y., Concept: M.K., N.Y., Design: M.K., B.S., N.Y., Data Collection or Processing: N.Y., Analysis or Interpretation: M.K., B.S., N.Y., Literature Search: N.Y., Writing: M.K., B.S., N.Y.

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

Financial Disclosure: The authors declared that this study received no financial support.

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