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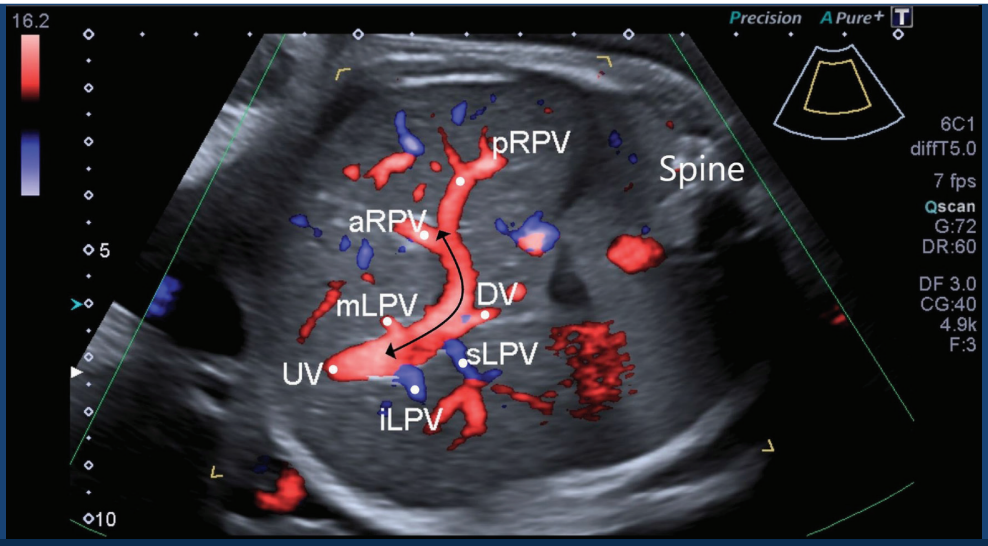

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Cover Picture: Cemil Gürses et al. Ductus venosus blood velocities

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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December. The publication language of the journal is English. Manuscripts are reviewed in accordance with "double-blind peer review" process for both reviewers and authors.

The target audience of Journal of the Turkish-German Gynecological Association includes gynecologists and primary care physicians interested in gynecology practice. It publishes original works on all aspects of obstertrics and gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor, diagnostic puzzle are also published. Suggestions for new books are also welcomed. Journal of the Turkish-German Gynecological Association does not charge any fee for article submission or processing.

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PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

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/),

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All manuscripts should be accompanied by an abstract. A structured abstract is required with original articles and it should include the following subheadings: Objective, Material and Methods, Results and Conclusion. A structured abstract is not required with review articles. The abstract should be limited to 250 words for original articles and review articles.

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Below the abstract provide 3 to 5 Keywords. Abbreviations should not be used as Keywords. Keywords should be picked from the Medical Subject Headings (MeSH) list (www.nlm.nih.gov/mesh/MBrowser.html).

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures. Provide information on informed consent and ethics committee approval.

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Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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Editorial

Dear Colleagues,

I am delighted to introduce the final issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2021. This issue is consisted of seven articles and two reviews that we hope you will read with interest. Visually appealing and informative figures are an essential part of scientific publication. You will read articles with eye-catching figures. Also you may have the oppurtunity to watch all of the latest videos here (http://www.jtgga.org/video).

Here we share some of our favorite articles that were published in this issue of the journal.

There is much evidence that oxidative stress is involved in the pathogenesis of preeclampsia. You will read an article investigating the association between the *SOD1* (Ins/Del) polymorphism and the risk of preeclampsia. You will also read an interesting study which developed an updated model to predict the risk of recurrence, based on the number of adverse pathologic features in women with International Federation of Gynecology and Obstetrics stage I uterine endometrioid carcinoma, who did not undergo any adjuvant treatment.

Polycystic ovary syndrome (PCOS) is associated with long-term health problems such as obesity, diabetes, hypertension, dyslipidemia, metabolic syndrome, and cardiovascular risk factors in reproductive age women. You will get the occasion to read the current situation of changes and comorbidities after reproductive age in patients with PCOS in the light of the latest evidence.

We would also like to remind you that time is rapidly approaching to our prestigious 14th Turkish-German Gynecology Congress which will be held in Antalya between May 28 and June 1 of 2022. As of before, our congress will be held to the highest scientific standards and we are working round the clock to optimize our traditionalized congress. At this year's congress we will be having lectures with the world's most reputable speakers; Prof. Jan Deprest (Fetal Medicine: In utero spina bifida repair), Prof. Thomas Ebner (Artificial reduction of the blastocoel before vitrification), Prof. Kutluk Oktay (Fertility preservation for ovarian insufficiency), Prof. Wolfgang Holzgreve (Non-invasive prenatal testing).

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Journal of the Turkish-German Gynecological Association's online manuscript submission and peer-review system has changed to "Manuscript Manager" which is a modern peer review tool for publishers in search of a budget-friendly manuscript submission system and a state-of-the-art peer review software solution. You will see how easy it is for you to sign up and submit an article.

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I would like to wish you a happy new year in 2022 and we are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TGGF

Quantification of recurrence risk based on number of adverse prognostic factors in women with stage I uterine endometrioid carcinoma

Andrew E. Cook[1], Ibrahim Aref[1], Charlotte Burmeister[2], Miriana Hijaz[3], Mohamed A. Elshaikh[1]

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Abstract

Objective: The goal was to develop an updated model to predict the risk of recurrence, based on the number of adverse pathologic features in women with International Federation of Gynecology and Obstetrics stage I uterine endometrioid carcinoma, who did not undergo any adjuvant treatment.

Material and Methods: Women at a single center who underwent surgical staging without adjuvant therapy between January 1990 and December 2019 were included. Cox proportional hazards model was used to identify independent predictors of relapse free survival (RFS). Prognostic groups were then created based on the number of independent predictors of recurrence that were identified (0, 1, or 2-3 risk factors). Overall survival (OS) and disease specific survival (DSS) were also calculated for each group.

Results: In total 1133 women were eligible for inclusion. Median follow-up was 84 months. Independent prognostic factors of recurrence included: age ≥60; grade 2 or 3 differentiation; and presence of lymphovascular space invasion (LVSI). Due to the small number of patients with either 2 or 3 risk factors, these groups were combined into one (group 2/3). Isolated vaginal cuff recurrence was the most common site of recurrence in all study groups (2%, 7%, and 17% for groups 0, 1, and 2/3, respectively). Five-year RFS rates were 96%, 85%, and 57% for groups 0, 1, and 2/3 ($p<0.01$), respectively. Five-year DSS rates were 99%, 96%, and 85% and 5-year OS rates were 94%, 85%, and 62% ($p<0.01$), respectively.

Conclusion: We identified older age, high grade, and presence of LVSI as independent predictors of recurrence for women with stage I uterine endometrioid carcinoma. Using these prognostic factors, recurrence risk can be quantified for individual patients, and these factors can be used in deciding the appropriate adjuvant management course. (J Turk Ger Gynecol Assoc 2021; 22: 262-7)

Keywords: Endometrial adenocarcinoma, recurrence, prognosis, survival

Received: 26 April, 2021 **Accepted:** 30 July, 2021

Introduction

Endometrial carcinoma (EC) remains the most commonly diagnosed gynecologic cancer in the United States (1). Most women present with early-stage disease, with excellent survival outcomes (2).

Hysterectomy is the standard of care for women with early-stage EC, resulting in most patients being cured. However, some groups of patients are at risk for cancer recurrence due to the presence of some pathologic risk factors. The Gynecologic Oncology Group-99 (GOG-99) study showed that women with some adverse pathologic features who did not undergo adjuvant treatment have a recurrence risk of 26% at 2 years. This trial solidified the classic definition of high-intermediate risk in stage I EC as follows: age ≥70 with one risk factor (grade 2 or 3 differentiation, presence of lymphovascular space invasion

Presented at the 2020 Annual American Society for Radiation Oncology (ASTRO) Meeting, October 2020.

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(LVSI), or deep myometrial invasion); age ≥50 with any two risk factors; and any age with all three risk factors (3). Women with only one of these risk factors experience lower recurrence rates of 5% at 5 years after adjuvant radiation therapy (RT) (4).

The National Comprehensive Cancer Network lists observation, RT, and chemotherapy as treatment options for patients with International Federation of Gynecology and Obstetrics (FIGO) stage I EC (5). Given the number of treatment options and their associated toxicities, determining recurrence risk for this group of patients is vital so that appropriate adjuvant treatments can be selected.

Outside of prospective trials, there have been several studies examining recurrence in solely early-stage patients with EC (6-21). However, some of these studies include a heterogenous group of women with non-endometrioid histologies, such as serous carcinoma, which are known to have a poor prognosis (8,10,11,21,22). All of these studies also include women who received various adjuvant treatments (6-21). Adjuvant therapy is known to significantly impact recurrence rates (3,23). Furthermore, some studies utilized nomograms, which can be cumbersome to use in daily clinical practice (7).

A simplified prognostic model for women with FIGO stage I EC was recently reported based on tumor grade, presence of LVSI, and percent myometrial invasion. While useful, the investigators reported that older age at diagnosis was not an independent risk factor for cancer recurrence (24).

In order to examine recurrence rates in women with FIGO stage I EC accurately, a population of women who did not undergo any adjuvant treatment is needed to answer this question. By including a larger study cohort with a longer follow-up, we sought to analyze independent predictors of recurrence in women with FIGO stage I uterine EC who did not receive any adjuvant therapies.

Material and Methods

After obtaining Henry Ford Institutional Review Board approval (approval number: IRB 4645), we searched our prospectively maintained database for women with 2009 FIGO stage I EC who underwent hysterectomy and no adjuvant therapy between January 1990 and December 2019. As this was a retrospective study, informed consent was waived. Exclusion criteria were the presence of synchronous malignancies and non-endometrioid histology. After surgery, patients underwent routine follow-up with surveillance testing performed as clinically indicated. Follow-up data, including timing of recurrence, was collected from patients' medical records when available. Patient demographics, surgical pathologic variables, and survival endpoints were collected. Baseline comorbidity burden (Charlson comorbidity score) was also collected for each patient immediately before hysterectomy.

Statistical analysis

The primary endpoint of the study was relapse free survival (RFS), which included both locoregional and distant recurrences. Univariate (UVA) and multivariate analyses (MVA) were first performed to determine independent predictors of RFS. Cox proportional hazards models were used to identify independent predictors of recurrence using a manual stepwise selection with an entry criterion of $p<0.2$ and stay criteria of $p<0.05$. Groups were then created based on the number of recurrence risk factors that were present. Then, for each of these groups, RFS, overall survival (OS), and disease specific survival (DSS) were determined using date of hysterectomy as the start date. Kaplan-Meier plots were generated for each of these outcomes. Nominal and numerical variables were analyzed using a chi-square test and Student's t-test, respectively. All analyses were performed in statistical software SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

After considering inclusion and exclusion criteria, a total of 1,133 patients were included with a median (range) follow-up of 86.8 (1-346) months. Table 1 summarizes baseline patient characteristics, pathologic findings, and recurrence patterns for the study cohort. The median age was 60 (26-93) years. There were 75 patients (6.6%) with FIGO stage IB disease. Pelvic lymph node (LN) dissection was performed in 59% (666 patients) with or without para-aortic lymphadenectomy. The median (range) number of LN examined was 2 (0-66). For some patients with adverse prognostic factors, it was not clear in the medical records why they did not receive the recommended adjuvant treatment. Patient refusal was the most commonly reported reason for not receiving adjuvant treatment. There was a total of 71 women (6%) who were diagnosed with cancer recurrence (pathologically, radiologically or both). The sites of their first relapse included isolated vaginal recurrence in 39/71 (54.9%), isolated pelvic recurrence in four (5.6%), vaginal and pelvic recurrence in nine (12.7%), isolated para-aortic recurrence in two (2.8%), and distant recurrence in 17 (23.9%) patients.

For the entire cohort, predictors of worse 5-year RFS on UVA were age (as continuous and dichotomous variables), higher grade, higher stage, and presence of LVSI. On MVA predictors were older age (≥60), grade 2/3, and presence of LVSI (Table 2). These three independent predictors were then used to design the final recurrence model. A score of 0 was applied if the following factors were present: age <60, grade 1, and lack of LVSI. A score of 1 was applied for the following risk factors: age ≥60, grade 2/3, and presence of LVSI.

Initially, four risks groups were created (group 0 with no risk factors, group 1 with one risk factor, group 2 with two risk

factors, and group 3 with three risk factors). Due to the small number of patients in groups with 2 or 3 risk factors, these were combined into one group (group 2/3), creating a total of three risk groups.

There were 871 patients (77%) in group 0, 220 patients (19%) in group 1, and 42 patients (4%) in group 2/3. Regarding baseline characteristics among the groups, there were significant differences noted in age, body mass index, characteristics of LN dissection, grade, lower uterine segment involvement, presence of LVSI, and stage (Table 1).

Five-year RFS rates for the three prognostic groups were 96% [95% confidence interval (CI) 95-97%] for group 0, 85% (95% CI 78-90%) for group 1, and 57% (95% CI 38-73%) for group 2/3 (p<0.01) (Figure 1). Independent predictors for RFS included age ≥60 years, higher tumor grade, and the presence of LVSI.

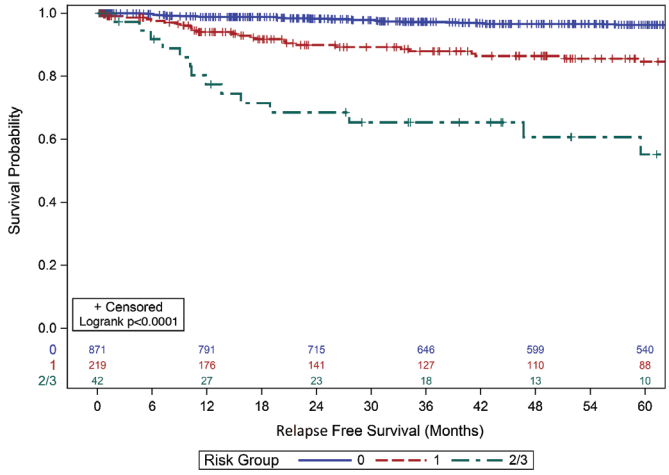

Figure 1. Kaplan-Meier plot comparing 5-year relapse free survival among risk groups

Table 1. Baseline patient characteristics across risk factor groups

Variable	Group 0 (n=871)	Group 1 (n=220)	Group 2/3 (n=42)	p
Age, median (range)	59.0 (26.0-91.0)	62.0 (30.0-92.0)	73.5 (34.0-93.0)	<0.01
Age <60	456 (52%)	89 (40%)	8 (19%)	<0.01
Age >60	415 (48%)	131 (60%)	34 (81%)	
Median (range) body mass index	36.2 (17.9-71.3)	33.4 (15.6-60.3)	30.5 (18.2-59.6)	<0.01
Median (range) Charlson Comorbidity score	0 (0-12)	0 (0-9)	0 (0-4)	0.27
Race				0.39
White	680 (78%)	159 (72%)	34 (81%)	-
African American	160 (18%)	53 (24%)	7 (17%)	-
Other	30 (3%)	8 (4%)	1 (2%)	-
FIGO stage IA	871 (100%)	176 (80%)	11 (26%)	<0.01
FIGO stage IB	0 (0%)	44 (20%)	31 (74%)	-
Tumor grade				<0.01
1	871 (100%)	63 (29%)	6 (14%)	-
2	0 (0%)	131 (60%)	21 (50%)	-
3	0 (0%)	26 (12%)	15 (36%)	-
Lymphovascular space invasion	0 (0%)	19 (9%)	28 (67%)	<0.01
Lower uterine segment involvement	83 (10%)	34 (15%)	10 (24%)	<0.01
Positive peritoneal cytology	12 (1%)	0 (0%)	1 (2%)	0.09
Lymph node dissection	470 (54%)	163 (74%)	33 (79%)	<0.01
Median number of nodes examined	1.0 (0.0-55.0)	5.0 (0.0-66.0)	4.5 (0.0-45.0)	<0.01
Median number of pelvic nodes examined	1.0 (0.0-41.0)	5.0 (0.0-44.0)	3.5 (0.0-36.0)	<0.01
Median number PA nodes examined	0.0 (0.0-32.0)	0.0 (0.0-25.0)	0.0 (0.0-17.0)	<0.01
Tumor recurrence	28 (3%)	29 (13%)	14 (33%)	<0.01
Site of first tumor recurrence				
Isolated vaginal recurrence	17 (2%)	15 (7%)	7 (17%)	0.23
Pelvic only recurrence	1 (0%)	3 (1%)	0 (0%)	0.65
Vaginal and pelvic recurrence	2 (0%)	4 (2%)	3 (7%)	0.35
PA only recurrence	0 (0%)	2 (1%)	0 (0%)	0.28
Distant recurrence	8 (2%)	5 (2%)	4 (9%)	0.67

FIGO: International Federation of Gynecology and Obstetrics, PA: Paraaortic

Five-year DSS rates for the three groups were 99% (95% CI 98-99%) for group 0, 96% (95% CI 91-98%) for group 1, and 85% (95% CI 66-93%) for group 2/3 (p<0.01) (Figure 2). Independent significant predictors for DSS included age ≥60 years and deep myometrial invasion.

Five-year OS for group 0 was 94% (95% CI 93-96%), 85% for group 1 (95% CI 78-89%), and 62% (95% CI 44-76%) for group 2/3 (p<0.01) (Figure 3). The independent predictors for OS were age ≥60, grade 2/3 differentiation, deep myometrial invasion, and high comorbidity burden.

Table 2 summarizes the results of MVA for the different survival endpoints including hazard ratios.

Discussion

Although prior studies have aimed at creating a method to predict recurrence for early-stage EC, a simplified model is needed that is based on women who were observed following surgery. Given the lack of data to answer this question, some investigators sought to develop a simplified risk stratification method for women with FIGO stage I EC with endometrioid histology who did not undergo any adjuvant therapy. The authors were able to include 976 patients and identified tumor grade 2/3, presence of LVSI, and stage IB as independent risk factors of recurrence, and three risk groups were established with 0, 1, or 2/3 risk factors (24). The current study further builds on these results, using a larger patient cohort and, importantly, longer follow-up.

We were able to determine that independent predictors of 5-year RFS were older age, high tumor grade, and presence of LVSI. Utilizing these predictors, we created three separate risk groups based on the number of factors present: 0, 1, and 2/3. The RFS survival endpoints for groups 0, 1, and 2/3 were 96%, 85%, and 57%. This model provides a more individualized approach to determine patients' recurrence risk based on the number of risk factors.

Traditionally, patients with early-stage EC were grouped into low-risk, intermediate-risk, high-intermediate risk, and high risk (3,23,25). These risk stratifications assume that all patients grouped into a specific category have the same risk of recurrence. This assumption is likely inaccurate given the definitions for each group are broad, leading to a heterogeneous cohort.

Also of note, our study confirms that the predominant pattern of first recurrence in women with early-stage EC is in the vagina. Overall, 39 patients (4%) experienced vaginal relapse across

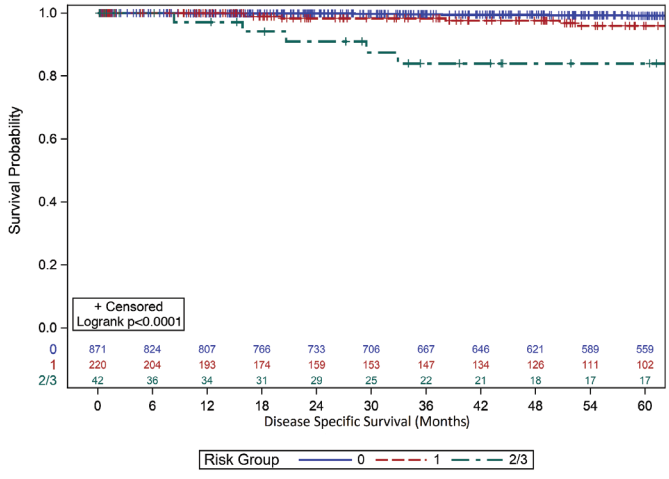

Figure 2. Kaplan-Meier plot comparing 5-year disease specific survival among risk groups

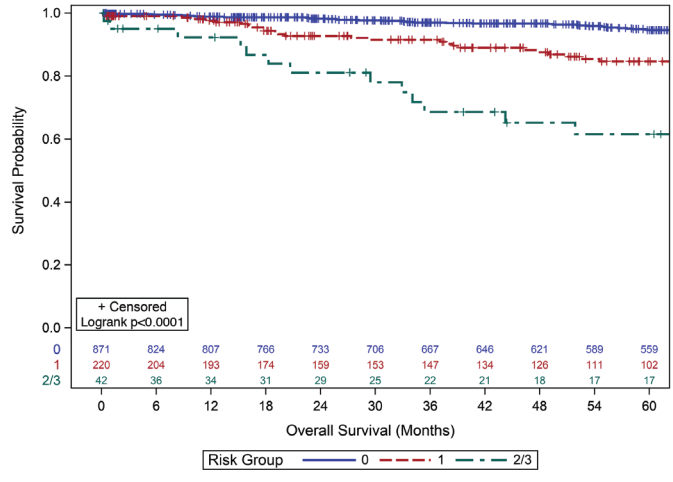

Figure 3. Kaplan-Meier plot comparing 5-year overall survival among risk groups

Table 2. Results of multivariate analyses of survival endpoints for the study cohort

	Relapse free survival			Disease specific survival			Overall survival		
Variable	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
Older age (>60 vs <60)	1.03	1.01-1.05	0.01	1.07	1.02-1.12	<0.01	1.07	1.04-1.09	<0.01
Tumor grade 2/3 vs grade 1	3.00	1.77-5.07	<0.01	2.53	0.90-7.10	0.08	2.36	1.44-3.88	<0.01
Positive lymphovascular space invasion	4.52	2.27-8.97	<0.01	2.12	0.54-8.34	0.28	1.72	0.83-3.60	0.15
FIGO stage IB vs IA	1.44	0.69-3.01	0.33	3.87	1.19-12.57	0.02	2.20	1.17-4.14	0.01
Charlson comorbidity score (as a continuous variable)	-	-	-	-	-	-	1.25	1.12-1.40	<0.01

HR: Hazard ratio, CI: Confidence interval, FIGO: International Federation of Gynecology and Obstetrics

all three risk groups. This finding is in agreement with a large prospective trial (23). This recurrence pattern helps to inform appropriate adjuvant treatment options.

The recurrence rates for our patient population are similar to those that have been identified in prospective studies. For patients with 0 risk factors, we found a 5-year RFS of 96%, which is similar to a study by Sorbe et al. (26) in which women who were deemed low-risk (FIGO stage IA-IB, endometrioid histology, and FIGO grade 1-2) underwent observation and were found to have a recurrence rate of 4.1% at 5 years. Our 5-year OS of 94% was also similar to this cohort, which was found to be 96.1% (26).

For our group with one risk factor, a comparable cohort was studied in the observation arm of Post Operative Radiation Therapy in Endometrial Carcinoma-1 (PORTEC-1), which included women with either <50% myometrial invasion and grade 2/3 disease or >50% myometrial invasion and grade 1-2 disease. Our 5-year RFS for group 1 was 85%, and this study showed a 5-year recurrence rate of 16%. Additionally, our 5-year OS was 85%, which was identical to the endpoint examined in this study (27).

Finally, our group with 2/3 risk factors can be compared to patients in the observation arm studied in GOG-99 (inclusion criteria described earlier). Our 5-year RFS was 57%, which was not as favorable compared to the 5-year recurrence rate of close to 30% in this study. The 5-year OS for our group of 57% is also notably lower than the OS of approximately 75% found in this study. One possible explanation for this discrepancy is that we were able to include only 42 patients in this group while the GOG-99 study observed 70 high-intermediate risk patients (3).

Additionally, as our study is retrospective, selection bias may explain the higher recurrence and lower survival in our patient cohort.

Of note, a dissimilar aspect between our study and many of the previously mentioned prospective studies is that myometrial invasion/stage was not found to be a significant predictor for recurrence in our analysis. Depth of myometrial invasion is a well-known predictor for recurrence, as demonstrated in the GOG-33 analysis (28). A possible explanation for us not identifying deep myometrial invasion as a predictor was that only 75 patients (6.6%) had FIGO stage IB disease. It is still likely that myometrial invasion predicts for recurrence and should be considered when determining treatment management.

Study limitation

Our study does have some other limitations. As noted above, this study is retrospective and prone to selection bias in terms of which treatment the patients received. Also, there were only 42 patients who were included in group 2/3. This small number of patients does limit the power of statistical analyses on this group and the conclusions that can be drawn from this data.

In addition to utilizing these risk factors to determine recurrence risk, a promising future method to help in this effort uses molecular prognostication. The Cancer Genome Atlas identified four molecular subgroups within EC: POLE ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high (29). PORTEC-4 is an ongoing study determining if these molecular groups are prognostic and if they can guide adjuvant treatment decisions (30). The results of this trial are eagerly awaited and may be practice changing.

Conclusion

This simplified recurrence model for patients with FIGO stage I EC includes three traditional independent predictors of RFS: older age, high tumor grade, and presence of LVSI. Given that risk groups defined by historical studies for this patient population are very heterogenous, this risk-scoring system can be applied to individual patients and is easy to utilize in daily clinical practice.

Ethics Committee Approval: *The study was approved by Henry Ford Institutional Review Board (approval number: IRB 4645).*

Informed Consent: *As this was a retrospective study, informed consent was waived.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: A.E.C., I.A., M.H., M.A.E.; Concept: I.A., M.H., M.A.E.; Design: I.A., M.H., M.A.E.; Data Collection or Processing: A.E.C., C.B.; Analysis or Interpretation: A.E.C., I.A., C.B., M.H., M.A.E.; Literature Search: A.E.C., M.A.E.; Writing: A.E.C., M.A.E.*

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

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Susceptibility to preeclampsia is associated with a 50-bp insertion/deletion polymorphism at the promoter region of the *SOD1* gene

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Abstract

Objective: There is much evidence that oxidative stress is involved in the pathogenesis of preeclampsia (PE). A 50 bp insertion/deletion (Ins/Del) functional polymorphism in the promoter region of *SOD1* has been reported. Due to a total lack of data, the aim of this study was to investigate the association between the *SOD1* (Ins/Del) polymorphism and the risk of PE.

Material and Methods: The current hospital-based case-control study included a total of 172 preeclamptic and 171 non-preeclamptic pregnancies. Genotyping was performed using the polymerase chain reaction method.

Results: Statistical analysis revealed that the Del/Del genotype significantly correlated with susceptibility to PE [odds ratio (OR): 6.53, 95% confidence interval (CI): 1.43-29.7, p=0.015]. Since maternal body mass index, family history of PE in first degree relatives, and educational levels were statistically associated with the susceptibility to PE, further analyses were carried out in order to estimate the adjusted ORs. After adjustment for aforementioned variables, the Del/Del genotype increased the risk of PE (OR: 5.98, 95% CI: 1.21-29.5, p=0.028).

Conclusion: The 50 bp Ins/Del in promoter region of the *SOD1* gene could be an intriguing susceptibility factor for developing preeclampsia in Iranian Caucasians. (J Turk Ger Gynecol Assoc 2021; 22: 268-72)

Keywords: Ins/Del, polymorphism, preeclampsia, susceptibility, superoxide dismutase

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Introduction

Preeclampsia (PE) affects approximately 5-8% of pregnancies and greatly contributes to morbidity/mortality of mother and fetus (1). Preeclampsia is defined as proteinuria and hypertension after 20 weeks of gestation. Furthermore, preeclamptic women are at risk for developing cardiovascular disease (2). The detailed etiology of PE remains unknown and thus precise prediction and prevention are difficult. Genetic components and environmental factors are known to be involved in the etiology of PE, and therefore PE is highly heritable (3,4). Many genetic association studies of candidate genetic polymorphisms have been performed to investigate the genetic background of PE (5).

Reactive oxygen species (ROS) lead to oxidation of numerous biomolecules, including DNA (6). Oxidative stress can occur due to an imbalance between production of ROS and antioxidant capacity. There is plentiful evidence that such conditions are implicated in the pathogenesis of PE (4,7-9). Antioxidant enzymatic system comprises a number of gene families, such as the family SODs [(EC 1.15.1.1) (EC 1.15.1.1) EC 1.15.1.1)]. In mammals, SODs have been classified into three different isoforms, including *SOD1* (MIM: 147450).

A 50 bp insertion/deletion (Ins/Del) in the *SOD1* promoter region has been reported (10). This is a functional polymorphism and alters gene expression with the Del allele resulting in lower *SOD1* mRNA levels (11). Association between the *SOD1*

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expression level and PE has been investigated (12). *SOD1* expression is significantly down-regulated under oxidative stress, for example it is decreased in morphine treated human SH-SY5Y cells (13), and in cells exposed to electromagnetic fields (14). *SOD1* Ins/Del genetic variation is related with complex human diseases associated with oxidative stress, such as breast and gastric cancers, age of onset of bipolar disorder, and cardiovascular disease (15-19).

We hypothesized that the Ins/Del genetic variation in *SOD1*, which is involved in cellular detoxification, may represent a good candidate for susceptibility to PE. Since no data has been published on the association between the *SOD1* (Ins/Del) polymorphism and the risk of PE, a hospital-based, case-control study was carried out and is presented here.

Material and Methods

Participants

This was a hospital-based case-control study. A total of 172 preeclamptic and 171 non-preeclamptic pregnancies, as healthy control group, were included in the study. The participants were recruited from the delivery ward at Zainabeieh, Hafez and Dena Hospitals (Shiraz, Fars province, south-west Iran). DNA extraction, genotype determination and statistical analysis were done in our research laboratory. The preeclamptic and non-preeclamptic women were matched with each other by age. PE was defined as persistent blood pressure above 140/90 mmHg and proteinuria of more than 0.3 g/24 hours, developing after 20 weeks of gestation. Exclusion criteria were twin pregnancies, fetal growth retardation, altered renal function, recurrent miscarriages, and placental abruption. The patients were not classified according to the severity of PE.

Body mass index (BMI, kg/m^2) was defined as weight (kg) divided by square of height (m^2). Data on age, family history of PE among first-degree relatives, educational level, gravidity, physical activity, interval from last pregnancy, and smoking habits were obtained through interviews with participants. As the gene pool of the Iranian population is heterogeneous (20,21), participants were selected from Caucasian Persian/Muslims living in Fars province, south-west Iran.

The minimum sample size that would be necessary to identify a significant difference in genotype distribution between PE and normal pregnancies was estimated using QUANTO software (http://biostats.usc.edu/foftware). Assuming 15% frequency for the D allele, equal number of cases and controls, Rg: 1.8, α=0.05, and β=0.80, minimally, 149 patients would be required. In the present study 172 PE and 171 normal pregnancies were included.

The current study was approved by the Ethical Committee of Shiraz University (approval number: SU.DB-9630578). This study was conducted in accordance with the Declaration of Helsinki. All pregnant women signed an informed consent before the study.

Genotyping

DNA was extracted from whole blood using a previously described method and stored at -20 °C until use (22). Genotyping analysis was carried out based on a polymerase chain reaction (PCR) method using specific primers as described previously (19). The PCR reaction was performed using 0.4 pmol/μL of each primer, 1.5 mM of $MgCl_2$, 0.2 mM each of desoxynucleotide triphosphates, 0.12 U/μL of Taq DNA polymerase (SinaGene, Iran) and genomic DNA as template. The reaction was pre-incubated for 5 minutes at 95 °C for denaturation, followed by 35 seconds at 95 °C, 30 seconds at 60 °C, and 35 seconds at 72 °C, for 31 cycles and a final extension at 72 °C for 10 minutes. The amplified DNA was electrophoresed in 1.5% agarose gel, under constant current of 90 volts for 40-60 minutes. Gels were stained and visualized by ethidium bromide staining under ultraviolet illumination.

Statistical analysis

Hardy-Weinberg equilibrium was investigated in the healthy controls and PE patients by χ^2 analysis. Continuous clinical characteristics were compared between the PE cases and non-PE controls, using Student's t-test. We performed unconditional logistic regression analysis to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) for assessment of differences in the prevalence of characteristics between PE cases and non-PE controls.

The following variables were used in the multivariate logistic regression analysis: maternal age (years); maternal prepregnancy BMI (kg/m^2); interval from last pregnancy (years); parity [nulliparous (0), multiparous (1)]; family history of PE in first degree relatives [negative family history (0), positive family history (1)]; smoking during pregnancy [no (0), yes (1)]; physical activity before pregnancy [no (0), yes (1)]; maternal educational level [high school or less (0), University (1)]; and the genotypes of the Ins/Del polymorphism [Del/Del (2), Del/Ins (1), and Ins/Ins (0)]. Statistical analyses were carried out using SPSS, version 25 software (IBM Inc., Armonk, NY, USA). Statistical significance was defined as p-value <0.05.

Results

Descriptive characteristics of the PE and control groups are shown in Table 1, 2, containing continuous and binary variables, respectively. Preeclamptic mothers compared to control subjects had significantly higher BMI (p<0.001) (Table 1). The mean interval from last pregnancy was statistically similar between normal pregnancies and PE outcomes. A positive history for PE among first degree relatives (OR: 8.25,

95% CI: 3.39-20.0, p<0.001) and higher educational levels (OR: 3.77, 95% CI: 2.01-7.08, p<0.001) were associated with risk of PE. However, neither smoking nor gravidity were associated with PE (Table 2). A higher percentage of controls had some history of smoking compared to the PE group, but due to the very low frequency of smokers, the difference was not significant.

Figure 1 shows the genotypes of the study polymorphism by electrophoresis of the PCR products on 1.5% agarose gel. The Ins/Del alleles have 297 and 247 bp, respectively. Table 3 summarizes the distribution of genotypes of the *SOD1* Ins/Del genetic variation in PE and normal pregnancies. The genotypes were in Hardy-Weinberg equilibrium in control subjects (χ^2=0.02, df=1, p=0.880). However, there was significant deviation between observed and expected genotypic frequencies in the PE group (χ^2=11.9, df=1, p<0.001). Statistical analysis indicated that the Del/Del genotype was significantly correlated with susceptibility to PE (OR: 6.53, 95% CI: 1.43-29.7, p=0.015). The Del allele increased the risk of PE (OR: 1.67, 95% CI: 1.08-2.58, p=0.020). The risk of PE significantly increased as a function of the number of Del alleles (χ^2 for trend: 4.75, p=0.029).

Since maternal BMI, family history of PE in first degree relatives, and educational levels were statistically associated with the risk of PE, further analyses were carried out in order to estimate the adjusted ORs. After adjustment for these variables, the Del/Del genotype significantly increased the susceptibility to PE (OR: 5.98, 95% CI: 1.21-29.5, p=0.028).

Table 1. Comparison of continuous characteristics between preeclampsia patients and healthy controls

Characteristics	Controls		PE		Results of comparisons		
	n	Mean ± SD	n	Mean ± SD	t	df	p
Age (years)	171	26.8±4.7	172	27.7±4.7	1.83	341	0.068
BMI (kg/m^2)	137	27.7±4.3	145	30.7±5.3	5.2	280	<0.001
Interval from last pregnancy (years)	87	4.6±3.1	77	5.3±4.2	1.22	163	0.222
SD: Standard deviation, PE: Preeclampsia							

Table 2. Comparison of binary characteristics between preeclampsia patients and healthy controls

Characteristics	Controls, n (%)	Cases, n (%)	OR	95% CI	p
Parity					
Nulliparous	75 (43.9)	90 (52.3)	1.0	-	-
Multiparous	96 (56.1)	82 (47.7)	1.40	0.91-2.14	0.117
Family history of PE in first degree of relatives					
No	161 (94.2)	130 (75.6)	1.0	-	-
Yes	6 (3.5)	40 (23.3)	8.25	3.39-20.0	<0.001
Missing data	4 (2.3)	2 (1.1)	-	-	-
Smoking habit					
No	157 (91.8)	168 (97.7)	1.0	-	-
Yes	7 (4.1)	4 (2.3)	0.53	0.15-1.85	0.324
Missing data	7 (4.1)	0 (0)	-	-	-
Physical activity					
No	147 (86.0)	142 (82.6)	1.0	-	-
Yes	21 (12.3)	29 (16.9)	1.43	0.77-2.62	0.249
Missing data	3 (1.7)	1 (0.6)	-	-	-
Maternal education levels					
High school and lower	154 (90.0)	125 (72.7)	1.0	-	-
University	15 (8.8)	46 (26.7)	3.77	2.01-7.08	<0.001
Missing data	2 (1.2)	1 (0.6)	-	-	-
Gender of fetus					
Males	83 (48.5)	83 (48.3)	1.0	-	-
Females	83 (48.5)	81 (47.1)	0.97	0.63-1.50	0.912
Missing data	5 (2.9)	8 (4.6)	-	-	-
OR: Odds ratio, CI: Confidence interval, PE: Preeclampsia					

Table 3. Association between the 50 bp Ins/Del genetic polymorphisms of the *SOD1* and susceptibility to preeclampsia

Genotypes/Alleles	Controls	Cases	OR	95% CI	p	OR*	95% CI	p
Ins/Ins	134	123	1.0	-	-	1.0	-	-
Ins/Del	35	37	1.15	0.68-1.94	0.597	1.15	0.60-2.22	0.658
Del/Del	2	12	6.53	1.43-29.7	0.015	5.98	1.21-29.5	0.028
Ins	303	283	1.0	-	-	-	-	-
Del	39	61	1.67	1.08-258	0.020	-	-	-

*: Adjusted odds ratio for maternal body mass index, family history of PE among first degree relatives, and educational level, OR: Odds ratio, CI: Confidence interval, PE: Preeclampsia

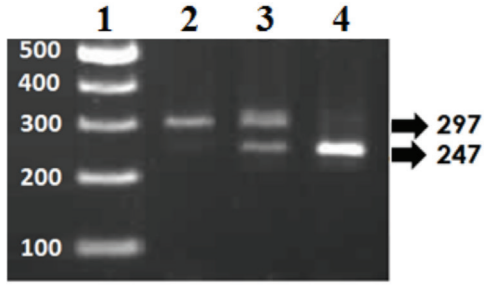

Figure 1. Polymerase chain reaction products of 50 bp insertion/deletion (Ins/Del) polymorphism at the promoter region of the *SOD1* gene. Lanes 1, 2, 3, and 4 are 100 bp DNA ladder, Ins/Ins, Ins/Del and Del/Del genotypes, respectively

Discussion

These results showed that maternal BMI, interval from last pregnancy, family history of PE among first degree relatives, and educational levels were significantly associated with PE, which were similar to previous studies (1,4,5,23-25).

It is well established that oxidative stress is associated with the pathogenesis of PE (4,7-9). SOD1 is involved in the antioxidant system and it has several genetic polymorphisms, including the 50 bp Ins/Del functional polymorphism in its promoter region (10). Investigation of the association between the *SOD1* Ins/Del functional polymorphism and the risk of PE was the main aim of this study. The Del allele is reported to significantly decrease promoter activity (11). Therefore, it was suggested that individuals having the Del/Del genotype would have lower antioxidant capacity compared to the Ins/Ins genotype (11). The Del/Del genotype showed positive correlation with susceptibility to PE, which supported our hypothesis. Previously, reduced expression of *SOD1* in peripheral blood mononuclear cells of PE patients had been reported (12). In addition, a significantly lower level of the SOD1 mRNA in trophoblast cells isolated from placentas of PE pregnancies has been reported (26,27). These reports are in keeping with the current findings. Investigation of the 50 bp Ins/Del genetic variation in the promoter region of *SOD1* may improve the prediction of susceptibility to PE and may aid in the design and development of new markers and treatment strategies.

The present case-control study has some limitations. First, it is well established that numerous environmental factors have significant association with the risk of PE (4,5). In the present study we did not investigate these factors alone and their possible gene-environment interactions. Second, patients were not classified according to the severity of PE. Third, the *SOD1* gene has other polymorphisms such as the A251G polymorphism (rs2070424) which is associated with some multifactorial traits (28-30). In the present study only the *SOD1* Ins/Del genetic polymorphism was investigated. It is highly likely that ethnicity may impact associations in complex human traits (31-33). Therefore, replication of the current study with a larger sample size and in other ethnic groups is suggested, as well as investigating the relationship between risk of PE and both environmental and genetic factors, simultaneously.

Conclusion

The 50 bp Ins/Del functional polymorphism in the promoter region of the *SOD1* gene appears to be an intriguing susceptibility factor for the development of preeclampsia in Iranian Caucasians. Further, larger studies are required to confirm and expand upon these findings.

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Ethical Committee Approval: *All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The current study was approved by the Ethical Committee of Shiraz University (approval number: SU.DB-9630578).*

Informed Consent: *Informed consent was obtained from all patients.*

Peer-review: *Externally peer-reviewed.*

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Differential expression of *Hsa-miR-517a/b* in placental tissue may contribute to the pathogenesis of preeclampsia

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Abstract

Objective: Preeclampsia (PE) is a pregnancy hypertensive disorder that affects both maternal and fetal health. Many studies have investigated possible mechanisms in the pathogenesis of PE although the role of the placenta is undeniable. Evaluation of placental-specific microRNAs may provide additional data about the pathogenic mechanism of PE. This study compared the expression levels of *Hsa-miR-517a/b* in placental tissues obtained from PE patients and healthy controls.

Material and Methods: One hundred tissues were obtained from fetal and maternal sides of the placenta of PE patients and healthy controls. Expression analysis was performed using quantitative real-time polymerase chain reaction.

Results: *Hsa-miR-517a/b* level was significantly decreased in PE compared to controls (expression ratio: 0.40; p=0.007). Down-regulation of *Hsa-miR-517a/b* was also detected in fetal-side placental samples when compared to maternal-side in PE (expression ratio: 0.33; p=0.04). Furthermore, decreased expression of *Hsa-miR-517a/b* was detected in fetal-side tissue from PE cases compared to fetal-side samples from healthy pregnancies (expression ratio: 0.36; p=0.03). In maternal-side placental samples the expression level did not differ between PE and healthy pregnancies (p=0.1).

Conclusion: These results demonstrate a differential expression of *Hsa-miR-517a/b* within placentas in pregnancies affected by PE and between placentas from PE and healthy pregnancies. Further studies are required to investigate a possible role for *Hsa-miR-517a/b* in the pathogenesis of PE. (J Turk Ger Gynecol Assoc 2021; 22: 273-8)

Keywords: Preeclampsia, microRNAs, expression analysis, placenta, *Hsa-miR-517a/b*

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Introduction

Preeclampsia (PE) is one of the most frequent complications of pregnancy and is characterized by high blood pressure and proteinuria after 20 weeks of gestation. About 2-8% of all pregnancies are affected by PE, which increases morbidity and mortality of fetus and mother (1,2). Despite much research the cause of PE remains unclear. However, different possible mechanisms have been proposed, including abnormality in trophoblast invasion, inappropriate placental implantation, ischemia, endothelial dysfunction, and imbalance between pro-angiogenic and anti-angiogenic factors (3-5). Imbalance in the components of the angiogenesis pathway in placental tissue is reported to be involved in PE pathogenesis (3). Vascular

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endothelial growth factor (VEGF) binds to its receptors, such as FMS-like tyrosine kinase 1, which results in the initiation of the angiogenesis pathway (6). Alterations in the mRNA levels of this ligand and its receptor have been previously investigated in PE (7-9). MicroRNAs (miRNAs) may play an important role in the regulation of expression of the genes related to the angiogenesis pathway (10). By binding to the 3' untranslated region of mRNAs, miRNAs regulate gene expression at the post-transcriptional level (11). MiRNAs transcribed from the chromosome 19 microRNA cluster (C19MC) are suggested to have a specific expression in trophoblast cells, and also in term and preterm preeclamptic placental tissue (12,13). Dysregulation of these placental-specific miRNAs could result in pregnancy-associated disorders, including PE (14). The *Hsa-miR-517* family contains three isoforms, including *Hsa-miR-517a*, *Hsa-miR-517b* and *Hsa-miR-517c*, all of which are transcribed from C19MC cluster. Due to the very close sequence similarity between *Hsa-miR-517a* and *b*, these two isoforms have been merged and are known as *Hsa-miR517a/b* (15,16). Previous studies regarding the expression level of the *Hsa-miR-517* family in the preeclamptic placental tissue showed inconsistent findings (12,17-19). As a fetomaternal organ, the placenta has fetal and maternal sides, and it is suggested that the expression of miRNAs may be different on each side (20,21). This characteristic of placental tissue has not usually been considered in previous studies. The aim of this study was to compare the expression levels of *Hsa-miR-517a/b* between preeclamptic and normal placenta. Moreover, the differential expression of *Hsa-miR-517a/b* was assessed in both the fetal-side and maternal-side of the placenta between both PE cases and healthy controls.

Material and Methods

Samples were collected from an equal number of PE patients and women with normal pregnancies. Placental tissues were collected from both the fetal-side and maternal-side of the placenta, up to ten minutes after delivery. On the fetal-side small placental tissue pieces were obtained, after separating the embryonic membranes, from just below the membranes and to a depth of less than 0.5 cm. On the maternal side, small biopsies of placenta were cut out from the cotyledons, also to a depth of less than 0.5 cm. The maternal-side specimens were obtained from the center of the cotyledons as far as possible from the calcified areas. All samples were washed with normal saline solution to remove debris and blood. The tissue samples were kept in RNA later solution (Ambion, Austin, Texas, USA) and stored at -20 °C until RNA extraction.

Both groups were Iranian with a common ethnic-geographic origin and were age-matched. Inclusion and exclusion criteria were considered based on the criteria defined by the American College of Obstetricians and Gynecologists for the diagnosis of PE (22). The PE women had systolic blood pressure above 140 mmHg and diastolic blood pressure above 90 mmHg, along with new-onset proteinuria, and no prepregnancy history of hypertension. Subjects with a history of hypertension, renal disease, and/or preexisting proteinuria, were not included in the study. All subjects signed written informed consent, and the Ethics Committee of the Shahid Beheshti University of Medical Sciences approved the study protocol (approval number: IR.SBMU.MSP.REC.1399.25).

According to the origin of biopsy samples, the placental tissues grouped into maternal preeclamptic (MP), fetal preeclamptic (FP), maternal control (MC), and fetal control (FC) samples. Using a RiboEx total RNA solution (GeneAll, Korea), the placental RNA was extracted according to the manufacturer's protocol. The High-Capacity complementary (cDNA) Reverse Transcription Kit (ABI, Cat. 4368814) was used to synthesize cDNA from 4 µg of isolated RNA. The stem-loop primer was designed using sRNAPrimerdb online software (available from; http://www.srnaprimerdb.com). The nucleotide sequence for the designed stem-loop primer was: GTCGTATCCAGTGCAGGG TCCGAGGTATTCGCACTGGATACGACACACTC. The synthesized cDNAs were stored at -70 °C.

Quantitative real-time PCR (qRT-PCR) was carried out to assess *Hsa-miR-517a/b* expression levels in the placental tissues using SYBR Green I Master Mix PCR (BioFACT™, Cat. DQ383-40h, Daejeon, Korea) in an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA). Thermal cycling parameters were: denature at 94 °C for 15 minute, and subsequent 50 amplification cycles including, 94 °C for 5 sec and then 60 °C for 34 sec. To evaluate the specificity of the PCR products, melting curve analysis and 2% agarose gel electrophoresis were performed. The primers' sequences were as follows: AAGCACATCGTGCATCCCT as the forward primer and GTCGTATCCAGTGCAGGGT as the universal reverse primer. *SNORD48* was used as the housekeeping gene. Specific primers used for amplification of *SNORD48* were: AACAGAAGAAGTGATGATGACCCCAGGTA as the forward and AATAATAATGTCAGAGCGCTGCGGTGAT as the reverse primer.

Statistical analysis

LinRegPCR software, version, 2017.1 (Academic Medical Center, Amsterdam, Netherlands) was used to specify the efficiency and the cycle threshold values for each qRT-PCR reaction. REST 2009 software (Qiagen, Hilden, Germany) was used to compare the expression level of the *Hsa-miR-517a/b* gene between the PE patients and healthy subjects. The *Hsa-miR-517a/b* expression was also compared between FP, FC, MP, and MC samples. The experimental data were analysed using Mann-Whitney U and Kruskal-Wallis tests in GraphPad Prism

software version 8.0 (GraphPad, La Jolla, CA, USA). A $p<0.05$ considered statistically significant.

Results

A total of 100 placental samples were examined, 50 from PE patients and 50 from women with normal pregnancies. Table 1 shows the clinical characteristics of the PE and control groups. Patients in the PE group had significantly higher systolic and diastolic blood pressure, and their babies had lower fetal birth weight and were born earlier. No significant differences were observed regarding mean age, mean body mass index, family history of hypertension and pregnancy loss between patients and controls.

Hsa-MiR-517 a/b was down-regulated in preeclamptic tissues compared to the control samples (expression ratio: 0.40; $p=0.007$) (Figure 1). A significant reduction was observed in the expression of *Hsa-miR-517a/b* in FP tissues compared to FC tissues (expression ratio: 0.36; $p=0.03$) (Figure 2). *Hsa-miR-517a/b* was also down-regulated in FP tissues compared to MP tissues (expression ratio: 0.33; $p=0.04$) (Figure 2). The similar difference was found when comparing the *Hsa-miR-517a/b* expression levels between FP and MC tissues (expression ratio: 0.189; $p=0.0002$) (Figure 2). The expression level was not statistically different between MP and MC tissues ($p=0.1$).

Discussion

The current study has shown that *Hsa-miR-517a/b* was down-regulated in the preeclamptic placenta compared to normal tissue. Moreover, it was observed that the dysregulation of *Hsa-miR-517a/b* was confined to the fetal side of the preeclamptic tissue. These result suggests that placenta, as a fetomaternal tissue, exhibits differential expression of genes on the fetal-side and maternal-side of the tissue.

In recent years, investigation into obstetrical and gynecological disorders, including PE, has increased. However, there are limited studies regarding the effect of miRNAs on gene expression regulation in this area (12,23). Placental-specific miRNAs are expressed uniquely in the placental tissue and have the potential to predict and act as biomarkers for placental conditions in both normal and adverse obstetrical pregnancy outcomes, including in PE (23). *Hsa-miR-517a/b* is a member of the C19MC miRNAs that locates on 19q13.42 and is considered placental-specific (14). Na et al. (18) reported that *Hsa-miR-517a/b* was down-regulated in the hydatidiform mole placenta when compared with the normal placenta. Preeclampsia and hydatidiform mole

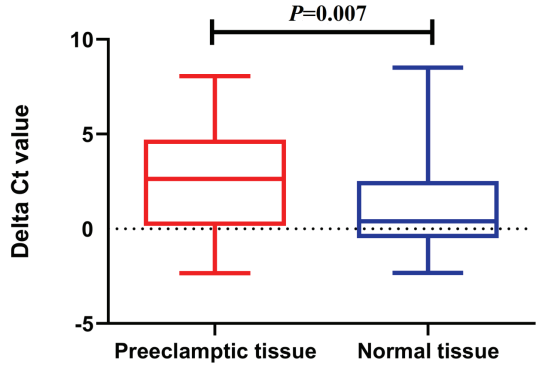

Figure 1. Comparison of the *Hsa-miR-517a/b* expression levels between preeclamptic tissues and normal tissues. The box plot represents the relative expression of *Hsa-miR-517a/b* normalized to *SNORD48* in preeclamptic placental tissues compared to normal placental tissues by Mann-Whitney U test. The Y and X axes show delta Ct (Ct_{target} gene-$Ct_{reference}$ gene) and studied groups, respectively. Boxes show the extent of the IQR and the central line is the median value. The whiskers show the full range of results. The median (IQR) values are 2.6 (0.2-4.7) and 0.4 (-0.5-2.5) in preeclamptic and normal tissues, respectively.

IQR: Interquartile range, Ct: Cycle threshold

Table 1. Basic characteristics and clinical features of the patients and controls

	PE patients	Controls	OR (CI)	p
Age (years)	32.4±5.0 (22-43)	33.2±6.9 (26-48)	-	0.14
Body mass index (kg/m²)	33.1±3.9 (25-40.1)	31.5±5.8 (20.8-41.7)	-	0.32
Gestational age at birth (weeks)	34.9±3.7 (32-39)	38.1±0.9 (36-40)	-	0.0005
Fetal weight (kg)	2.6±0.9 (1.9-3.9)	3.2±0.5 (1.8-3.9)	-	0.03
Systolic blood pressure (mmHg)	161±6 (120-190)	111±7 (90-120)	-	<0.0001
Diastolic blood pressure (mmHg)	92±9 (80-120)	73±8 (60-90)	-	<0.0001
Family history of hypertension (%)	44	24	2.5 (0.7-8.4)	0.14
History of pregnancy loss (%)	32	16	2.5 (0.6-9.6)	0.19
Preeclampsia				
Mild (%)	64	-	-	-
Severe (%)	36	-	-	
PE: Preeclampsia, OR: Odds Ratio, CI: Confidence interval				

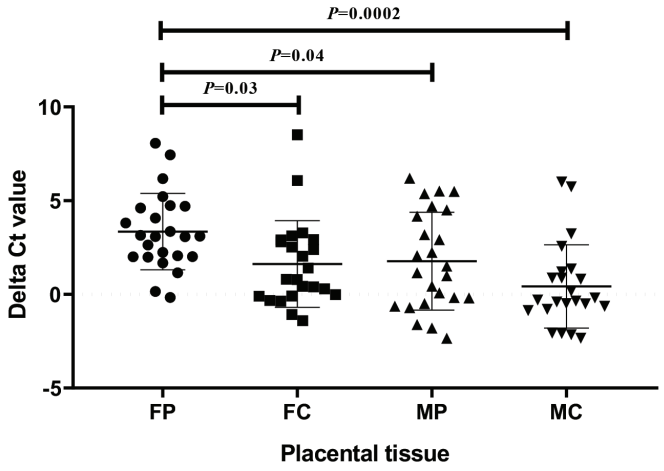

Figure 2. Comparison of *Hsa-miR-517a/b* expression levels between the fetal side of preeclamptic tissues (FP), fetal side of control tissues (FC), maternal side of preeclamptic tissues (MP) and maternal side of control tissues (MC). The Y and X axes show delta Ct (Ct_{target} gene-$Ct_{reference}$ gene) and placental tissues, respectively. The median (IQR) values are 3.1 (2-4.7), 0.8 (-0.1-2.9), 1.5 (-0.5-4.6), and -0.3 (-0.8-1.2) in FP, FC, MP, and MC groups, respectively.

FP: Fetal preeclamptic, FC: Fetal control, MP: Maternal preeclamptic, MC: Maternal control, Ct: Cycle threshold

placenta originate from an inappropriate invasion of trophoblast cells (24-26). Zhu et al. (19) reported the differential expression of miRNAs in placental tissues from PE patients vs normal controls. They showed that several miRNAs located at 19q13.42, including *Hsa-miR-517*, *Hsa-miR-518b*, and *Hsa-miR-519e* were expressed differentially in preeclamptic placentas (19). Anton et al. (12) also reported that *Hsa-miR-517a/b* and *Hsa-miR-517c* play an important role in PE development via the regulation of placental and trophoblastic function. In a recent study, Hromadnikova et al. (17) investigated the association between differential expressions of 15 different C19MC miRNAs in the placenta and pregnancy-related complications, including PE, fetal growth restriction, and gestational hypertension. They showed that *Hsa-miR-517* was down-regulated in all these pregnancy complications, including PE (17). They previously reported that *Hsa-miR-517-5p* was increased in the maternal plasma of preeclamptic women, and therefore suggested that up-regulation of C19MC miRNAs serves as a characteristic phenomenon of established PE (27). Down-regulation of *Hsa-miR-517a/b*, as shown in the present study, could contribute to the dysregulation of predicted target genes and, therefore, to the pathogenesis of PE. According to the TargetScan database, *STAT1*, *FOXC1,* and *HOXA5* are predicted as *Hsa-miR-517a/b* target genes (28). These genes are involved in the conduction of important biological pathways that includes angiogenesis. In some cases, PE is associated with inhibition of the angiogenesis pathway due to the imbalance between pro-angiogenic and anti-angiogenic factors (29). Signal transducer and activator of transcription 1 (STAT1), as a transcription factor, regulates the expression of several genes via the interferon-gamma (IFN-γ)/STAT1 pathway, involved in inflammation and angiogenesis, the main features of PE. A bioinformatics analysis conducted by Luo et al. (30) proposed STAT1 as a hub gene in the protein-protein interaction network related to PE. Zhang et al. (31) showed that *STAT1* is expressed under conditions of hypoxia and affects the expression of *VEGF-A* and *HIF1a* in glioma cells. In a similar fashion to these tumor cells, altered expressions of placental *VEGF* and *HIF1a* are associated with the pathogenesis of PE. Moreover, in a recent study, it was suggested that IFN-γ/STAT1 promotes the expression of erythropoietin-producing hepatocellular receptor B4 that regulates endothelial activation in PE pathogenesis (32).

The Forkhead box C1 (FOXC1) belongs to the FOX transcription factor family and plays a vital role in embryonic development. A previous study suggested that FOXC1 affects angiogenesis by regulating the balance between anti- and pro-angiogenic pathways. It was shown that *FOXC1*-null mutations in mice result in over-expression of *sFlt1* as an inhibitor of angiogenesis (33). Løset et al. (34), in a genome-wide transcriptional profiling study, confirmed the differential expression of *FOXC1* between the preeclamptic and normal decidual tissues. *HOXA5* is an anti-angiogenic homeobox gene. Increased expression of *HOXA5* is associated with decreased expression of pro-angiogenic genes such as *VEGFR2*, and increased expression of *thrombospondin-2* (*TSP2*) as an angiogenesis inhibitor (35). In most previous gene expression studies in preeclamptic tissues, it is unclear whether the tissue is obtained from the maternal or fetal side of the placenta. Therefore, these discrepant results may be due to tissue sampling from different placental zones.

In the present study, it was hypothesized that gene expression levels might differ between the maternal and fetal sides of the placenta, due to the effects of the maternal and fetal genomes, respectively. To the best of our knowledge, there are very few articles regarding this issue. Sahay et al. (36) showed the differential VEGF and VEGFR1 protein levels in different regions of the placenta. The present results provide robust evidence of the differential expression of *Hsa-miR-517a/b* in the maternal and fetal sides of the placenta.

Conclusion

PE was associated with down-regulation of *Hsa-miR-517a/b*. Furthermore, *Hsa-miR-517a/b* expression level was different only in the fetal side of placental tissue when comparing between PE patients and healthy controls. The results of the present study may help to understand the possible mechanisms

involved in the pathogenesis of PE. Moreover, the present results confirmed the importance of tissue sampling accuracy when undertaking gene expression studies in PE.

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Ethics Committee Approval: *The Ethics Committee of the Shahid Beheshti University of Medical Sciences approved the study protocol (approval number: IR.SBMU.MSP.REC.1399.25).*

Informed Consent: *All subjects were informed about the study and each gave written consent.*

Peer-review: *Externally peer-reviewed.*

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Early-cleavage versus blastocyst stage embryo transfer: a prospective comparative study

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Abstract

Objective: To evaluate whether or not embryo transfer (ET) day has an effect on the rates of clinical pregnancy (CPR) and live birth (LBR) in in vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI) treatment.

Material and Methods: A total of 757 patients who underwent IVF-ICSI treatment between 2012 and 2017 were included. The participants were stratified into three groups according to ET day: group 1 (day 2 transfer, n=43); group 2 (day 3 transfer, n=633); and group 3 [day 5 (blastocyst) transfer, n=81]. Basal parameters and IVF-ICSI outcomes were compared between the groups.

Results: Group 1 and 2 patients were older, had a higher body mass index, worse response rate, lower antral follicle count, lower peak estradiol levels, and less endometrial thickness, and required higher total gonadotropin dose than group 3. In addition, the number of oocytes and metaphase II oocytes, fertilization rate, and 2 pronucleus number were statistically different between the groups. The CPR (19.5% vs 36.9% vs 39.0%, respectively) and LBR (14.6% vs 30.4% vs 35.1%, respectively) were significantly lower in group 1 than in groups 2 and 3 ($p<0.05$). Grade 1 embryos were significantly more prevalent in groups 1 and 2 with clinical pregnancy positive [odds ratio (OR): 4.444; 95% confidence interval (CI): 0.876-22.536; p=0.001 and OR: 1.756; 95% CI: 1.234-2.500; $p<0.001$) and live birth (OR: 5.021; 95% CI: 0.787-31.768; p=0.001 and OR: 1.676; 95% CI: 1.154-2.433; p=0.007).

Conclusion: These data suggest that an earlier ET day has a negative effect on the CPR. Older primary infertile women should not postpone their desire to have a baby because they appear to be poorer responders. (J Turk Ger Gynecol Assoc 2021; 22: 279-85)

Keywords: Assisted reproductive techniques, clinical pregnancy rate, embryo transfer day, ovulation induction

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Introduction

The last stage of in vitro fertilization-intracytoplasmic (IVF-ICSI) treatment is the transfer of embryos, which result from the fertilization of oocytes with sperm, following controlled ovarian stimulation (COH) into the endometrium (1,2). Embryo transfer (ET) is an important step in assisted reproductive technology, and although day 2 or day 3 transfer of early-cleavage embryos is widely preferred clinically, blastocyst ET is gaining attention because it provides better synchronization between embryo and endometrium as well as high-quality embryo presentation (3,4).

Early-cleavage ET of four- or eight-cell embryos on day 2 or 3, respectively, may be advantageous to embryonic survival in terms of requiring less in vitro time (5,6). There are two key reasons for the widespread adoption of this form of ET; first, the development of the embryos is slower, and second, embryos placed in the endometrium at this stage are more likely to survive (2). However, as a result of accelerating advances in blastocyst culture over recent decades, ET has shifted from the early-cleavage period to this later stage (7). A number of studies have reported that the synchronization between embryo and endometrium in the blastocyst stage increased implantation success and, consequently, the rates of clinical pregnancy and

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live birth (8,9). There are also studies that report no appreciable difference (2,10). The aim of this study, therefore, was to investigate whether or not ET day had an effect on CPR and LBR in IVF-ICSI treatment.

Material and Methods

Study participants and data collection

This prospective study was carried out at Ali Kemal Belviranlı Maternal Women's Health and Children's Hospital, IVF Unit. The outcome of fresh ICSI cycles occurring between January 2012 and December 2017 were reviewed. Inclusion criteria were participants aged 20-44 years, body mass index (BMI) between 18 and 35 kg/m^2, regular menstrual cycles, no uterine abnormalities on ultrasound, and normal baseline hormonal levels. Participants were excluded from the study if they were ≥45 years, BMI ≥35 kg/m^2, and if any significant concurrent illness or metabolic disorder was present.

Ethical board approval was given from the Necmettin Erbakan University Faculty of Medicine Institutional Review Board (approval number: 2011/57). Written and oral informed agreement was obtained from the participants.

Data items collected included age (years), BMI (kg/m^2), smoking status, infertility period, cause of infertility, the baseline (day 3) follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) levels, concentrations of thyroid-stimulating hormone (TSH) and prolactin, and antral follicle count, stimulation parameters, IVF-ICSI outcome and CPR.

Ovarian stimulation and oocyte retrieval

Controlled ovulation stimulation was performed using the agonist [long luteal, gonadotropin-releasing hormone agonist (GnRHa) or microdose flare-up protocol] or the flexible gonadotropin-releasing hormone antagonist (GnRHant) protocol.

The GnRHa protocol: First, pituitary down-regulation was performed with a GnRH agonist. Then, the ovaries were stimulated by exogenous gonadotropins. The GnRH agonist leuprolide acetate (Lucrin; Abbott Cedex, İstanbul, Turkey) was administered subcutaneously daily from day 21 of the preceding luteal phase (0.5 mg/day) until menstruation, and then the dose was decreased to 0.25 mg/day until ovulation was triggered. Recombinant FSH (Puregon; Organon, Oss, the Netherlands, or Gonal F; Serono, İstanbul, Turkey) was used for stimulation. The initial gonadotropin dose used was individualized according to the patient's age, baseline serum FSH concentration on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first three days (100-225 IU recombinant FSH/day). Thereafter, the dose of gonadotropin was adjusted according to the individual ovarian responses, which were monitored by measuring serum E_2 levels and transvaginal ultrasonography (LOGIC 200 PRO, General Electric, Seoul, South Korea). Ovulation was triggered by the administration of 250 IU recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Serono, İstanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved 36 h after the hCG injection, and ICSI was performed for all IVF-ET patients.

Microdose flare-up protocol: Recombinant FSH (Puregon; Organon, Oss, the Netherlands, or Gonal F; Serono, İstanbul, Turkey) and the GnRH agonist leuprolide acetate (Lucrin; Abbott Cedex, İstanbul, Turkey) were both administered subcutaneously and daily (0.5 mg/day, subcutaneously for five days) on day 3 of a withdrawal bleed following at least three weeks of oral contraceptive use. The initial gonadotropin dose used was individualized according to the patient's age, baseline serum FSH concentration on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first three days (100-225 IU recombinant FSH/day). Thereafter, the dose of gonadotropin was adjusted according to the individual ovarian responses, which were monitored by measuring serum E_2 levels and transvaginal ultrasonography (LOGIC 200 PRO, General Electric, Seoul, South Korea). Ovulation was triggered by the administration of 250 IU recombinant hCG (Ovitrelle, Serono, İstanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved 36 h after the hCG injection, and ICSI was performed for all IVF-ET patients.

The GnRHant protocol: Pituitary down-regulation was achieved and maintained using the flexible GnRHant protocol. Recombinant human FSH (r-FSH; Gonal-F, Merck-Serono, or Puregon, MSD) or human menopausal gonadotropin (hMG; Menogon or Menopur; Ferring) was used for COH. The initial gonadotropin dose used for ovarian stimulation was individualized according to the patient's age, baseline serum FSH concentrations on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first three days (150-225 IU r-FSH/day), and thereafter, the gonadotropin dose was adjusted according to the individual's ovarian response. Serial estrogen levels and two-dimensional follicle measurements by transvaginal ultrasonography (LOGIC 200 PRO, General Electric, Seoul, South Korea) were performed. A daily dose of 0.25 mg of GnRHant (Cetrotide, Merck-Serono, or Orgalutran, MSD) was initiated when the leading follicle diameter was ≥13 mm or the serum E_2 level reached ≥300 pg/mL. When at least two dominant follicles reached dimensions of 18 mm or greater in diameter, hCG (250 µg, Ovitrelle, Merck-Serono, İstanbul, Turkey) was administered, and oocytes were retrieved 36 hours after the hCG injection. ICSI was then applied in accordance with our clinical procedures.

Embryo grading and ET procedure

Embryos were classified according to a simplified system based on Veeck's morphological criteria: grade 1 embryos have equal-sized blastomeres and no cytoplasmic fragmentation; grade 2 embryos have blastomeres of equal size and minor cytoplasmic fragmentation covering ≤10% of the pre-embryo surface; grade 3 embryos have blastomeres of distinctly unequal size and variable fragmentation; grade 4 embryos have blastomeres of equal or unequal size and moderate-to-significant cytoplasmic fragmentation covering >10% of the pre-embryo surface; and grade 5 embryos have few blastomeres of any size and severe fragmentation covering ≥50% of the pre-embryo surface. None of the embryos were classified as grade 5 in this study. Blastocyst quality was categorized as excellent (AA), good (AB, BA, BB), fair (BC, CB) or poor (CC), on the basis of trophectoderm and inner-cell-mass quality scores (11). The highest quality embryos were selected for ET on days 2, 3, and 5 after fertilization. The number of embryos transferred (two or fewer per patient) complied with national regulations in Turkey.

Two senior physicians performed ultrasonographic-guided (Logiq 200 Pro, General Electric, Seoul, South Korea) ETs using an ET catheter system. A sterile speculum was introduced to the vagina in the lithotomy position and the vagina and the cervix were cleared using sterile cotton swabs.

An embryologist loaded the embryos into a soft transfer catheter which was advanced to the ET physician who deposited the embryos approximately 10 mm from the uterine fundus under ultrasound (USG) imaging. The catheter was gently removed after 5 seconds. In cases of ET with external guidance, an initial catheter with inner sheath was inserted into the external cervical os, and then advanced through the cervical canal and internal os to 10 mm of the uterine fundus using USG. The internal sheath was withdrawn, and a second catheter loaded with embryos was introduced in its place and advanced to approximately 10 mm from the uterine fundus where the embryos were deposited. Difficult transfers required the use of a stylet in addition to this form of external guidance. All catheters were immediately checked for retained embryos, blood, and the patient remained in the Trendelenburg position for about 10 minutes. Patients in whom tenaculum were excluded from the study. Luteal phase support was provided with progesterone in the form of Crinone 8% gel (Serono, İstanbul, Turkey) at a daily dose of 90 mg. Baseline parameters and IVF-ICSI outcomes were compared between the groups. The subjects were categorized into three groups according to ET day: group 1 (day 2 transfer); group 2 (day 3 transfer); and group 3 [day 5 (blastocyst) transfer]. Basal parameters, clinical and laboratory IVF-ICSI outcomes, and pregnancy rates were compared between the groups.

Statistical analysis

The statistical analyses were performed using SPSS, version 15.0 for Windows (SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used to investigate normality of distribution of data sets. For comparison of data between groups, ANOVA was used with normally distributed variables and Kruskal-Wallis test was used with non-parametric data. Categorical data were examined by Pearson's chi-square test, and Fisher's exact test was applied if the expected frequency was <5 in >20% of all cells. Continuous variables are presented as the mean ± standard deviation and categorical variables are reported as number of cases and percentages. Bonferroni-adjustment was used to control the type 1 errors for all possible multiple comparisons. Logistic regression analyses were used to evaluate the factors thought to affect CPR and LBR. A $p<0.05$ value was assumed to indicate statistical significance.

Results

A total of 808 patients underwent IVF-ICSI during the study period. Fifty-one patients were excluded from the study, specifically those with age ≥45 years (n=19), BMI ≥35 kg/m^2 (n=14), systemic disease (n=9), endocrine or metabolic disorders (n=6), and concomitant medication (n=3). The remaining 757 participants were classified into the three ET groups [group 1 (n=43), group 2 (n=633) and group 3 (n=81)] and their outcomes analyzed (Figure 1).

A comparison of the sociodemographic and stimulation characteristics of the participants is provided in Table 1. No differences were evident between the groups in terms of smoking status, infertility period, cause of infertility, baseline FSH, LH, E_2, TSH, prolactin levels, duration of stimulation, stimulation protocol, progesterone levels, and endometrial thickness on hCG administration ($p>0.05$). Groups 1 and 2 patients tended to be older, have a higher BMI, worse responder have lower antral follicle count, lower peak E_2 levels, and less endometrial thickness, and required an increased total gonadotropin dose than group 3.

The laboratory and reproductive outcomes of the participants are summarized in Table 2. While the ET technique was comparable between the groups ($p>0.05$), the numbers of oocytes retrieved, metaphase II (MII) oocytes, 2 pronucleus (2PN) number, fertilization rate, and the rate of grade 1 embryos per woman decreased in groups 1 and 2 ($p<0.05$). The CPR (19.5% vs 36.9% vs 39.0%, respectively) and LBR (14.6% vs 30.4% vs 35.1%, respectively) were significantly lower in group 1 than in group 2 and group 3 ($p<0.05$).

Logistic regression analysis of the factors thought to affect CPR and LBR are given in Table 3. Grade 1 embryos in groups 1 and group 2 were significantly more likely to result in clinical pregnancy positive [odds ratio (OR): 4.444; 95% confidence

interval (CI): 0.876-22.536; p=0.001 and OR: 1.756; 95% CI: 1.234-2.500; p<0.001] and live birth (OR: 5.021; 95% CI: 0.787-31.768; p=0.001 and OR: 1.676; 95% CI: 1.154-2.433; p=0.007).

Discussion

Patients in group 1 and 2 were older, had a higher BMI, worse responder rate, lower antral follicle count, lower peak E_2 levels, less endometrial thickness, and required an increased total gonadotropin dose than the other transfer day groups. In addition, the number of oocytes and MII oocytes, 2 PN, fertilization rate, and grade 1 embryos were statistically different between the groups and the CPR was lower in group 1 than in group 2 and group 3.

Conventional early-cleavage ET on day 2 or 3 is thought to be the most suitable approach in terms of intrauterine microenvironment for the survival of embryos used in IVF-ICSI treatment (12). With this form of ET, embryos will spend less time in vitro (2). Two reasons why early-cleavage stage ET is widely accepted in IVF-ICSI treatment are the low embryonic growth rate in the culture environment and their survival rate once placed in the uterus (13). In the selection of embryos to be transferred in the early-cleavage stage, the number of blastomeres, fragmentation rate, and morphological appearance are assessed, and genomic activation and gene transcription are limited according to the blastocyst. As such, it is possible to overlook chromosomal anomalies (14,15).

Rapid developments in blastocyst culture over the last two decades have prompted a shift to day 5 or day 6 ET in many clinics (2), although the debate about the perinatal outcomes of either approach continues. Whilst improved success has been reported in blastocyst over early-cleavage ET in the literature (8,9), a significant difference was not found in a number of other studies (2,10). Since cell compaction and genomic activation are beyond the control of maternal RNA by the 5th day, culture media are enriched by the addition of organic and inorganic material to ensure the longer survival of the embryos (16).

Blastocyst ET has two potential advantages over an early-cleavage approach in that this later stage physiologically overlaps better with the intrauterine microenvironment and it allows the more accurate selection of the embryos that are most likely to survive (17). In early-cleavage ET, the intrauterine microenvironment has been seen to stress the embryos and reduce implantation success (18). In addition, uterine contractility is lower in the blastocyst period, and so the expulsion rate of transferred embryos is reduced (19). Considering the possibility of embryonic arrest in the blastocyst stage, embryologists must conduct careful evaluation and the

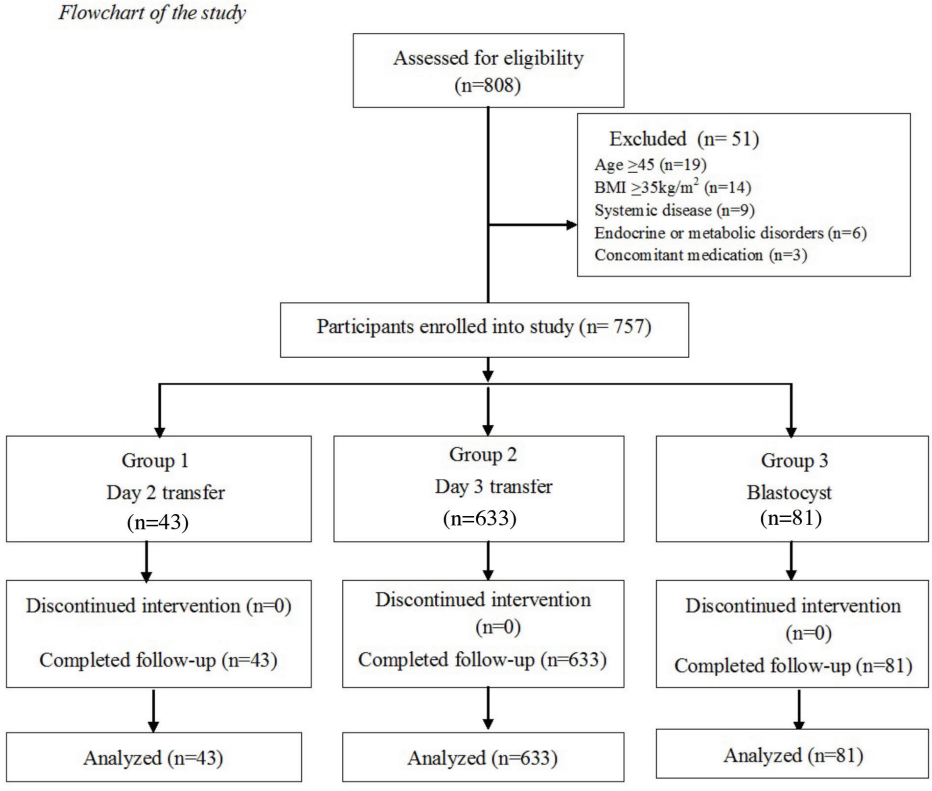

Figure 1. Enrolment and follow-up of study subjects

BMI: Body mass index

Table 1. Demographic and stimulation characteristics of the patients

		Day 2 transfer group 1 (n=43)	Day 3 transfer group 2 (n=633)	Day 5 transfer group 3 (n=81)	p		
					1 vs 2	1 vs 3	2 vs 3
Age (years)		32.12±5.65	29.68±4.52	28.36±4.33	0.003	<0.001	0.046
Age >40 (years) (%)		4.5%	1.6%	1.3%	0.323	0.323	0.323
BMI (kg/m^2)		27.76±4.31	25.89±4.53	25.21±4.86	0.030	0.011	0.433
Smoking rate (%)		4.9%	7.9%	3.9%	0.324	0.324	0.324
Duration of infertility (years)		7.21±4.45	5.97±3.40	5.95±3.48	0.085	0.085	0.085
Etiology of infertility (%)	Male factor	26.8%	37.5%	35.1%	-	-	-
	Tubal factor	2.4%	2.2%	1.3%	0.012	<0.001	0.021
	Unexplained	26.9%	39.1%	55.8%	-	-	-
	Poor responder	43.9%	21.2%	7.8%	-	-	-
Baseline-FSH (IU/mL)		7.41±2.87	7.08±2.28	6.57±1.97	0.109	0.109	0.109
Baseline-LH (IU/mL)		5.13±2.39	5.53±2.87	6.20±3.37	0.119	0.119	0.119
Baseline-estradiol (pg/mL)		40.82±16.94	44.22±15.95	46.55±18.74	0.189	0.189	0.189
Antral follicle count		5.35±2.37	6.51±2.50	7.78±2.21	0.026	0.001	0.045
TSH (µIU/mL)		2.44±0.96	2.17±1.13	2.17±1.09	0.317	0.317	0.317
Prolactin (ng/mL)		16.02±7.90	16.09±8.62	18.69±12.21	0.058	0.058	0.058
Stimulation protocol (%)	Long	26.8%	19.1%	26.0%	-	-	-
	Antagonist	73.2%	80.1%	74.0%	-	0.327	-
	Microdose	0.0%	0.8%	0.0%	-	-	-
Duration of stimulation (days)		10.12±1.40	9.74±1.54	9.78±1.61	0.304	0.304	0.304
Gonadotropin dose (IU)		2567.68±1193.01	1948.55±834.94	1708.05±829.89	0.006	<0.001	0.043
Estradiol levels on day hCG (pg/mL)		1499.49±691.37	1903.98±1199.97	2741.39±1265.31	0.003	<0.001	<0.001
Progesterone levels on day hCG (pg/mL)		0.85±0.43	0.81±0.38	0.91±0.40	0.078	0.078	0.078
Endometrial thickness on day hCG (mm)		9.80±1.77	10.22±1.66	10.44±1.74	0.151	0.151	0.151
Endometrial thickness on transfer day (mm)		9.64±1.55	1070±1.88	10.91±2.26	0.007	0.017	0.744

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone, hCG: Human chorionic gonadotropin

Table 2. Laboratory and reproductive outcome parameters of the patients

		Day 2 transfer group 1 (n=43)	Day 3 transfer group 2 (n=633)	Day 5 transfer group 3 (n=81)	p		
					1 vs 2	1 vs 3	2 vs 3
Number of oocytes retrieved		5.71±3.91	9.23±5.36	13.55±5.66	<0.001	<0.001	<0.001
Number of MII oocytes		3.95±2.46	7.18±4.11	11.19±5.05	<0.001	<0.001	<0.001
2 pronucleus		2.07±1.27	4.69±3.04	8.03±3.56	<0.001	<0.001	<0.001
Fertilization rate (%)		61.17±26.99	68.59±24.01	74.36±19.30	0.129	0.012	0.043
Grade 1 embryo (%)		34.1%	64.7%	97.4	<0.001	<0.001	<0.001
The embryo transfer technique (%)	Easy transfer with a soft catheter	24.4%	21.2%	15.6%	0.704		
	After external guidance transfer	68.3%	72.2%	75.3%			
	Difficult transfer with a stylet	7.3%	6.6%	9.1%			
Clinical pregnancy rate (%)		19.5%	36.9%	39.0%	0.039	0.028	0.710
Live birth rate (%)		14.6%	30.4%	35.1%	0.033	0.021	0.434

MII: Metaphase II

Table 3. Logistic regression analysis of the factors thought to affect clinical pregnancy and live birth rates

		Clinical pregnancy			Live birth rate		
		OR	95% CI	p	OR	95% CI	p
Age (years)	Day 2 transfer	0.985	0.857-1.133	0.834	0.970	0.828-1.137	0.709
	Day 3 transfer	0.941	0.906-1.107	0.057	0.956	0.919-1.033	0.382
	Day 5 transfer	0.929	0.829-1.040	0.399	0.948	0.846-1.062	0.354
Grade 1 embryo (%)	Day 2 transfer	4.444	0.876-22.536	0.001	5.021	0.787-31.768	0.001
	Day 3 transfer	1.756	1.234-2.500	<0.001	1.676	1.154-2.433	0.007
	Day 5 transfer	-	0.0-0.0	0.999	-	0.0-0.0	0.999
Number of oocytes retrieved	Day 2 transfer	1.052	0.856-1.266	0.606	1.123	0.924-1.364	0.244
	Day 3 transfer	1.044	0.913-1.076	0.156	1.048	0.916-1.082	0.103
	Day 5 transfer	1.088	0.998-1.186	0.055	1.084	0.994-1.181	0.068
Number of MII oocytes	Day 2 transfer	1.010	0.738-1.383	0.750	1.104	0.796-1.532	0.554
	Day 3 transfer	1.076	0.934-1.120	0.341	1.087	0.943-1.133	0.059
	Day 5 transfer	1.070	0.979-1.181	0.131	1.069	0.973-1.174	0.165
Gonadotropin dose (IU)	Day 2 transfer	0.956	0.906-1.010	0.445	0.954	0.886-1.114	0.377
	Day 3 transfer	0.974	0.979-1.105	0.820	0.904	0.896-1.046	0.092
	Day 5 transfer	0.990	0.909-1.055	0.725	0.924	0.909-1.011	0.187

MII: Metaphase II, OR: Odds ratio, CI: Confidence interval

most suitable embryos should be left to day 5 or day 6 for ET. Otherwise, the IVF-ICSI cycle may be need to be canceled because of the likelihood of developmental cessation (20).

One study randomized 243 IVF-ICSI cycles across day 2, day 3, and blastocyst ET, and while there was no difference between the groups in terms of CPR, the miscarriage rate was higher in the blastocyst transfer patients (4). Elsewhere, although transfers of blastocyst embryos have returned higher live birth rates as compared to early-cleavage ET, no significant difference was observed in terms of cumulative pregnancy rates (2). According to a meta-analysis of 13 randomized controlled studies, blastocyst ET partially increases CPR and live birth rate and causes no change in multiple pregnancy or miscarriage rates as compared to the early-cleavage approach (2). However, findings regarding cumulative pregnancy rates are insufficient, and more data is needed to clarify this issue.

In the present study, the mean age and BMI of the early-cleavage group were higher than the blastocyst ET patients. Ovarian reserve rates were lower, and so the number of oocytes retrieved, MII oocytes, 2 PN and grade 1 embryos, and the fertilization rate, were all lower in this group. Early-cleavage ET therefore had to be applied in this group as the quality of embryos developed had parameters that would adversely affect the success of the IVF-ICSI treatment.

The strength of the current study includes its prospective arrangement, the adequate number of subjects in each group, and the prototypical sample from central Turkey; the results can be generalized to most of the country's population. However, the potential limitations of the study are that it was conducted in a tertiary care institution and that the cumulative CPR was not evaluated because no frozen ETs were included.

Conclusion

These results show that an earlier ET day has a negative impact on CPR. Older infertile women should not postpone their desire to have a baby because they are poor responders, and it should be explained that the chances of successful treatment are lower. Further studies with more participants are needed to clarify this situation.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Local Ethics Committee of the Necmettin Erbakan University Faculty of Medicine (approval number: 2011-57).*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: H.A.İ., Z.Ö.İ.; Concept: H.A.İ.; Design: Z.Ö.İ.; Data Collection or Processing: H.A.İ., Z.Ö.İ.; Analysis or Interpretation: H.A.İ., Z.Ö.İ.; Literature Search: H.A.İ., Z.Ö.İ.; Writing: H.A.İ., Z.Ö.İ.*

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Fetal left ventricular modified myocardial performance index and renal artery pulsatility index in pregnancies with isolated oligohydramnios before 37 weeks of gestation

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Abstract

Objective: The aim was to evaluate fetal left modified myocardial performance index (Mod-MPI) and renal artery Doppler parameters in fetuses with isolated oligohydramnios and a normal amount of amniotic fluid.

Material and Methods: This was a prospective cohort study; 25 pregnancies with isolated oligohydramnios and 25 healthy, gestational age-matched controls, between 24+0 to 36+6 weeks of gestation, were recruited. Primary outcome was to compare left modified MPI and mean fetal renal artery pulsatility index (PI). The secondary outcome was to compare adverse perinatal outcomes between the groups.

Results: Mean Mod-MPI was significantly higher (p=0.001) and isovolumetric relaxation time was longer (p=0.009) in the isolated oligohydramnios group. Mean renal artery PI values were not different between the groups. Birthweight (p=0.041) and gestational age at birth (p=0.001) were significantly lower, and incidences of delivery before 37 weeks (p=0.034) and Cesarean section due to non-reassuring fetal heart rate testing (p=0.021) were significantly higher in women with isolated oligohydramnios than the control group. We found no significant relationship between Mod-MPI and adverse perinatal outcomes.

Conclusion: Fetuses with isolated oligohydramnios have increased left Mod-MPI, which may be due to mild cardiac diastolic dysfunction. Increased Mod-MPI is not associated with adverse perinatal outcomes and does not seem to help in the management of pregnancies before 37 weeks of gestation with isolated oligohydramnios. (J Turk Ger Gynecol Assoc 2021; 22: 286-92)

Keywords: Isolated oligohydramnios, myocardial performance index, perinatal outcome, preterm, renal artery Doppler

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Introduction

Amniotic fluid volume assessment is a standard part of obstetric sonography and abnormalities of the amniotic fluid volume are related to increased risk for adverse perinatal outcomes (1). An amniotic fluid index (AFI) ≤5 cm or a single vertical pocket of amniotic fluid ≤2 cm is the most common definition for oligohydramnios. Approximately 0.5% to 5% of pregnancies are complicated by oligohydramnios (2). Oligohydramnios without fetal structural and chromosomal abnormalities, intrauterine infection, uteroplacental insufficiency, premature rupture of membranes and known maternal disease is known as isolated oligohydramnios (3). According to the literature, obstetrical outcomes of pregnancies with isolated oligohydramnios are controversial. Some studies have shown that isolated oligohydramnios is a predictor of adverse outcome (4,5) while others have not confirmed the association (3,6).

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Fetal cardiac function can be evaluated non-invasively by measuring Doppler-derived myocardial performance index (MPI) (7). Lower variability and higher inter- and intra-observer agreement were obtained with improved modification of myocardial performance index (Mod-MPI) (8). Higher left Mod-MPI values were demonstrated to be associated with left ventricular dysfunction (9). Left Mod-MPI has been investigated in a number of complicated pregnancies to evaluate fetal cardiac dysfunction (10-12). To the best of our knowledge, there is no study focusing on fetal cardiac function in isolated oligohydramnios.

The main component of amniotic fluid is fetal urine in the second and third trimester. Thus, decreased amniotic fluid volume can be seen secondary to impaired fetal renal function (13). Renal arterial perfusion affects urine output directly, which can be measured with renal artery Doppler parameters. However, results of studies focusing on alteration in renal Doppler parameters in isolated oligohydramnios were not in agreement and so it is difficult to state that isolated oligohydramnios is associated with renal artery Doppler parameters (14-16).

The aim of this study was to investigate whether pregnancies before 37 weeks of gestation with isolated oligohydramnios differ in measures of fetal cardiac function and renal artery flow velocity waveforms from those with pregnancies with normal amniotic fluid. The outcome of pregnancies between these two groups was also compared and the value of the left Mod-MPI in the prediction of adverse perinatal outcomes was evaluated.

Material and Methods

This prospective cohort study was conducted between August 2017 and June 2018 in the İstanbul University unit at Cerrahpaşa, in the Cerrahpaşa Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Perinatology. After ethical approval (İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethical Committee (approval number: 215728), twenty-five pregnancies with isolated oligohydramnios between 24+0 to 36+6 weeks of gestation were recruited. An AFI ≤5 cm was defined as oligohydramnios. These subjects were matched with 25 healthy, gestational age-matched singleton pregnancies with normal amniotic fluid (AFI: 8-25 cm). Exclusion criteria included: multiple pregnancies; preeclampsia; pregestational or gestational diabetes; other chronic diseases; premature rupture of membranes; fetal growth restriction; fetal infection; structural or chromosomal abnormalities; and pregnancies with pathological umbilical and uterine artery Doppler indices. During the study period, Voluson E10 C4-8-D (GE Healthcare, Zipf, Austria) curved and convex array probes were used.

After informed consent was obtained, a detailed fetal anatomy examination was performed and AFI was calculated using the 4-quadrant method (1). In the apical four-chamber view, the Doppler sample gate was kept at 3 mm and located on the left wall of the ascending aorta, between the leaflets of the aortic valves (AV) and mitral valves (MV). The angle of insonation was between 0 and 300, and the fastest Doppler sweep (15 cm/s) was used. Isovolumetric contraction time (ICT) was measured from MV closure to AV opening. Ejection time (ET) was measured from AV opening to closure. Isovolumetric relaxation time (IRT) was measured from AV closure to MV opening (Figure 1). The Mod-MPI was calculated using the formula (ICT + IRT)/ET. Three consecutive measurements were performed and the mean value was used for calculation. To evaluate renal artery blood flow, coronal plane was obtained and the Doppler gate was located on the proximal third of the renal arteries (Figure 2). The angle of insonation was close to 0°. Three consecutive measurements were made for the calculation of mean left and right renal artery pulsatility index (PI).

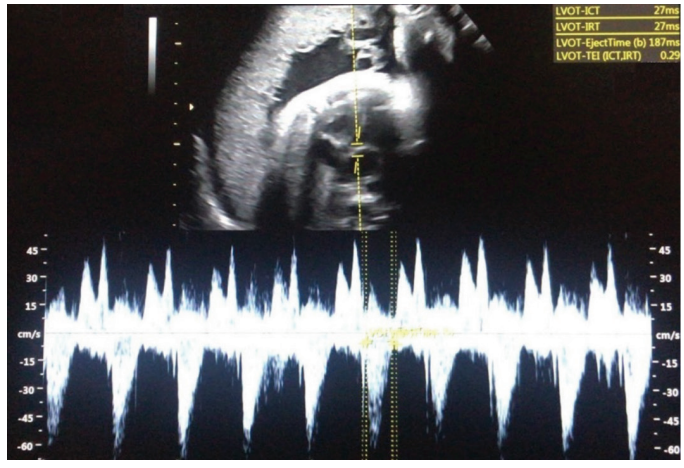

Figure 1. Measurement of fetal left ventricle myocardial performance index

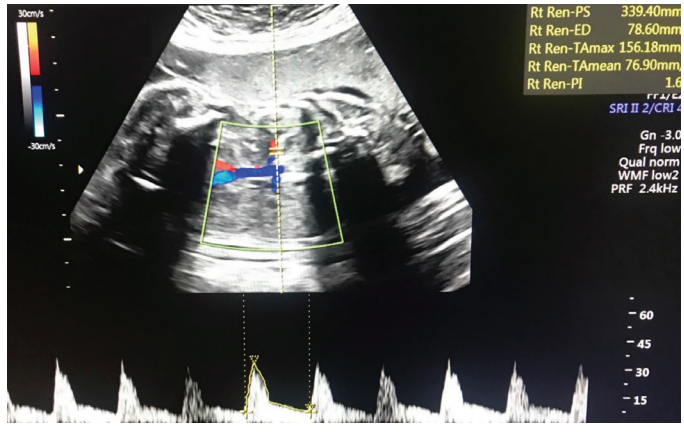

Figure 2. Measurement of fetal renal artery Doppler

Pregnancies with isolated oligohydramnios were followed every 2-3 weeks with umbilical artery Doppler until 32 weeks of gestation and every 1-2 weeks with non-stress test (NST) and umbilical artery Doppler until 36 weeks of gestation and twice weekly thereafter. Persistently non-reactive NST was used as an indication for delivery. Labor was induced in isolated oligohydramnios cases at 39 weeks gestation if not delivered before. Pregnancies with normal amniotic fluid were followed every four weeks until 36 weeks of gestation and examined at 38 weeks and 39 weeks of gestation and twice weekly thereafter until 41 weeks of gestation. Labor was induced at 41 weeks gestation if not delivered before. The follow-up algorithm for patients is shown in Figure 3. Route of delivery, gestational age at birth, birth weight and the presence of meconium-stained amniotic fluid at delivery were evaluated. Primary outcome was to compare left Mod-MPI and mean fetal renal artery PI and secondary outcome was to compare adverse perinatal outcomes between the groups. Adverse perinatal outcomes were defined as: umbilical cord arterial pH <7.2, Apgar 5 min <7, cesarean delivery for non-reassuring fetal heart-rate testing, transient tachypnea of the newborn (TTN), respiratory distress syndrome (RDS), admission to the neonatal intensive care unit (NICU), hypoxic ischemic encephalopathy (HIE), and neonatal death.

Statistical analysis

Statistical analysis was made with SPSS version 20.0 (IBM Inc., Armonk, NY, USA). Categorical data were analysed using the chi square test or Fisher's exact test. Numerical variables were compared using Student's t-test or Mann-Whitney U test. Pearson's rank correlation was used to assess the relationship between MPI and adverse perinatal outcomes. A p-value <0.05 and r>0.5 were considered to be statistically significant.

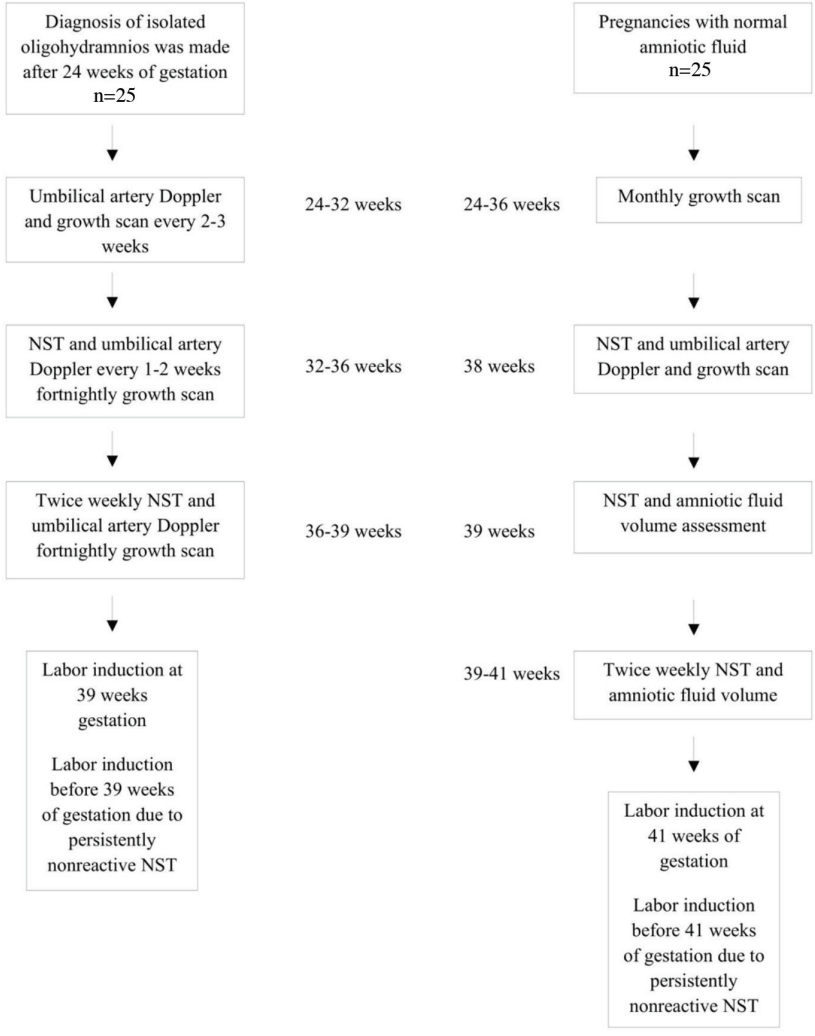

Figure 3. Follow-up algorithm for patients

NST: Non-stress test

Results

The clinical characteristics of pregnacies with isolated oligohydramnios and normal amniotic fluid are illustrated in Table 1. Maternal age and nulliparity showed no difference. Gestational age at ultrasound and umbilical artery PI values were similar between the two groups. Unsurprisingly, mean AFI was significantly different at 31 mm and 109 mm in the isolated oligohydramnios and control group, respectively (p<0.001).

Cardiac and renal artery Doppler parameters of pregnancies with isolated oligohydramnios and control group are presented in Table 2. Mean Mod-MPI value was significantly higher and IRT was longer in the isolated oligohydramnios group than the control group (p=0.001 and p=0.009, respectively). Mean ICT, ET values and mean renal artery PI value did not differ between the isolated oligohydramnios and control groups.

Perinatal outcomes in the isolated oligohydramnios and control group are presented in Table 3. Gestational age at birth and birth weight were significantly lower (p=0.041 and p=0.001, respectively), and incidences of delivery before 37 weeks and cesarean section due to non-reassuring fetal heart rate testing were significantly more common (p=0.034 and p=0.021, respectively) in women with isolated oligohydramnios than the control group. Incidences of labor induction, arterial cord blood pH <7.2, Apgar 5 min <7, meconium stained amniotic fluid, admission to NICU, TTN, HIE, RDS and neonatal death were not significantly different between the groups. Preterm induction of labor was performed in two patients with isolated oligohydramnios due to non-reassuring fetal heart rate at 36-37 weeks gestation. There was no significant correlation between Mod-MPI value and adverse perinatal outcomes (Pearson's p=0.112, p=0.440).

Discussion

In the current study, it was observed that pregnancies with isolated oligohydramnios before 37 weeks were characterized by a higher rate of preterm delivery and a higher rate of cesarean delivery for non-reassuring fetal heart rate testing during labor. However, adverse perinatal outcomes such as arterial cord pH <7.2, Apgar 5 min <7, meconium-stained amniotic fluid, admission to NICU and neonatal death were not significantly different between the pregnancies with isolated oligohydramnios and normal amniotic fluid volumes. Although we observed a higher rate of preterm delivery in the isolated oligohydramnios, the neonatal morbidity was similar. One possible explanation for this discrepancy might be the higher rate of late preterm deliveries at a gestational age of 36-37 weeks. Although it has been reported that isolated oligohydramnios at preterm pregnancy is associated with higher rate of adverse neonatal outcomes, subgroup analysis showed that adverse outcomes were substantially related to the induction of delivery but not to the isolated oligohydramnios itself (6). Melamed et al. (6) showed that the neonatal outcome in the isolated oligohydramnios group (expectant-management) was similar to that observed in the control group.

Table 1. The clinical characteristics of pregnancies with isolated oligohydramnios and control group

	Isolated oligohydramnios	Control group	p
N	25	25	-
Maternal age (years)	30.6±6.3	29.8±5.4	0.668
Nulliparity	14 (56)	12 (48)	0.778
Gestational age at ultrasound (weeks)	30.2±4.8	30±5	0.83
Mean AFI (mm)	31 (21-46)	109 (90-140)	<0.001
Umbilical artery PI	0.99±0.14	0.99±0.17	0.907
Data are presented as mean ± standard deviation or n (%), AFI: Amniotic fluid index, PI: Pulsatility index			

Table 2. Cardiac and renal artery Doppler parameters of pregnancies with isolated oligohydramnios and control group

	Isolated oligohydramnios	Control group	p
N	25	25	-
Modified myocardial performance index	0.53±0.11	0.43±0.08	0.001
Isovolumetric contraction time (ms)	36.6±0.9	32.1±0.7	0.069
Ejection time (ms)	161.7±19.4	169.8±10.9	0.074
Isovolumetric relaxation time (ms)	48.6±1.0	41.2±0.9	0.009
Mean renal artery PI	2.31±0.41	2.31±0.23	0.995
Data are presented as mean ± standard deviation, PI: Pulsatility index			

Table 3. Perinatal outcomes in the isolated oligohydramnios and control group

	Isolated oligohydramnios	Control group	p
N	25	25	-
Gestational age at birth (weeks)	36.9±2.7	38.4±2.1	0.041
<37 weeks	8 (32)	2 (8)	0.034
<34 weeks	2 (8)	1 (4)	0.552
Birth weight (g)	2660±570	3199±464	0.001
Labor induction	6 (24)	2 (8)	0.123
Cesarean delivery	14 (56)	7 (28)	0.045
Non-reassuring fetal heart rate	7 (28)	1 (4)	0.021
Meconium-stained amniotic fuid	0	0	N/A
Arterial cord pH <7.2	0	0	N/A
5-min Apgar score <7	3 (12)	0	0.07
Neonatal intensive care unit admission	4 (16)	1 (4)	0.157
Transient tachypnea of the newborn	2	0	0.149
Respiratory distress syndrome	2	1	0.552
Hypoxic ischemic encephalopathy	0	0	N/A
Neonatal death	0	0	N/A
Data are presented as mean ± standard deviation or n (%), N/A: Not applicable			

A recent meta-analysis including six studies of isolated oligohydramnios in 27,526 women showed that pregnancies with isolated oligohydramnios had significantly higher rates of meconium aspiration syndrome, cesarean delivery for fetal distress and admission to the NICU. It was concluded that isolated oligohydramnios is a pathological finding and there are not sufficient data to determine the optimal timing of delivery to reduce the risk of adverse outcomes (5). Several other studies have reported that perinatal outcomes in pregnancies with isolated oligohydramnios were similar to pregnancies with normal amniotic fluid (17,18). Our findings support the studies reporting that isolated oligohydramnios is not significantly associated with adverse perinatal outcome (6,17,18).

The amount of amniotic fluid depends on renal filtration and urine production. Renal artery Doppler velocimetric parameters reflect the arterial perfusion of the kidneys and these parameters may be related to amniotic fluid level. Several studies investigated the relationship between fetal renal artery Doppler parameters and amniotic fluid level but the results of these studies were inconsistent (14,15,19,20). Yoshimura et al. (20) showed that renal artery PI is significantly higher in pregnancies with isolated oligohydramnios compared to pregnancies with normal amniotic fluid. In another study, including 63 pregnancies followed from 16 to 41 weeks, there was no correlation between amniotic fluid levels and fetal renal artery PI (14). Benzer et al. (15) evaluated renal artery PI in pregnancies with oligohydramnios at 22, 28 and 34 weeks of gestation and found significantly higher renal artery PI only at 28 weeks of gestation, but not at 22 or 34 weeks. Budunoglu et al. (16) reported that renal artery PI was not significantly different between patients with isolated oligohydramnios and normal amniotic fluid at 25 to 40 weeks of gestation. In the current study, renal artery PI was not significantly different between pregnancies before 37 weeks of gestation with isolated oligohydramnios and normal amniotic fluid, suggesting that isolated oligohydramnios may not be related to impaired renal artery blood flow.

Mod-MPI values are associated with fetal left ventricular function and it has become a reliable marker of fetal cardiac dysfunction. Fetal MPI has been evaluated in several high-risk pregnancies, such as diabetic and postterm pregnancies, fetal growth restriction and twin-twin transfusion syndrome (12,21,22). We evaluated Mod-MPI in pregnancies with isolated oligohydramnios and demonstrated subtle cardiac dysfunction in fetuses with isolated oligohydramnios compared with healthy controls. The higher Mod-MPI levels have been found to be primarily due to an elevated IRT. IRT becomes abnormal in the initial stages of cardiac dysfunction and is mainly caused by decreased diastolic compliance (23). Calcium reuptake of myocardial cells are reduced, which leads to prolongation of complete cardiomyocyte relaxation and an increased IRT (24). Based on the findings of the present study, we speculate that there may be a mild diastolic dysfunction in fetuses with isolated oligohydramnios. However, whether oligohydramnios is the cause *per se* or the consequence of mild diastolic dysfunction

is not clear. There is only one study that has evaluated the association between an increased volume of amniotic fluid and Mod-MPI in pregnancies with isolated polyhydramnios. In that study, Mod-MPI was significantly higher in isolated polyhydramnios compared with controls and Mod-MPI was also associated with adverse perinatal outcome (25). However, although Mod-MPI was significantly increased in isolated oligihydramnios pregnancies in our study, we could not demonstrate any association between Mod-MPI values and adverse perinatal outcomes in fetuses with isolated oligohydramnios. Our study suggests that Mod-MPI evaluation does not seem to help in the management of pregnancies before 37 weeks of gestation with isolated oligohydramnios.
The strength of this study was its prospective design and measurement of Doppler parameters by a single experienced examiner. However, the limitation of the study was the small sample size.

Conclusion

Fetuses with isolated oligohydramnios have increased Mod-MPI which may be due to mild fetal cardiac diastolic dysfunction. Increased Mod-MPI was not associated with adverse perinatal outcomes in fetuses with isolated oligohydramnios. Further large studies are needed to investigate the importance of Mod-MPI in isolated oligohydramnios.

Ethics Committee Approval: *The study was approved by the Ethical Committee of the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine (approval number: 215728).*

Informed Consent: *It was obtained.*

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The efficacy of dydrogesterone use to suppress premature luteinizing hormone surge on cycle outcomes in controlled ovarian stimulation

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Abstract

Objective: Progestins are used as an alternative to gonadotropin releasing hormone (GnRH) antagonists to suppress premature luteinizing hormone (LH) surge and a flexible protocol has been defined recently. The aim of this study was to compare the efficacy of flexible protocols with dydrogesterone and GnRH antagonist in suppressing LH surge.

Material and Methods: This retrospective, case-control study, was conducted in an infertility unit of a tertiary university hospital. A daily dose of 40 mg dydrogesterone was compared with GnRH antagonist (GnRHant) in controlled ovarian hyperstimulation cycles between July 2018 and July 2019. Dydrogesterone was started when the leading follicle was 12 mm or serum estradiol was over 300 pg/mL. A subgroup analysis of poor responder patients was also performed.

Results: In total there were 105 subjects aged between 23 and 41 years, 52 in the dydrogesterone group and 53 in the GnRHant group. Duration of pituitary suppression was longer in dydrogesterone group. Premature ovulation was observed in 11.5% (6/52) and 0% in the dydrogesterone and GnRHant groups, respectively. However, collected oocyte counts and metaphase II oocyte counts were found to be similar between the groups. The six patients with premature ovulation were in poor responder subgroup.

Conclusion: Dydrogesterone can be used as an alternative to antagonist regimen in patients where embryo transfer is not planned in the same cycle. However, flexible regimen may not be appropriate in patients with diminished ovarian reserve, as advanced follicular maturation and delayed suppressive effect of oral progesterone may cause premature ovulation. Randomized controlled trials in particular patient groups are required to determine the most effective minimum dose and time of application to ensure treatment success. (J Turk Ger Gynecol Assoc 2021; 22: 293-9)

Keywords: Controlled ovarian hyperstimulation, dydrogesterone, luteinizing hormone surge, progestin-primed ovarian stimulation

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Introduction

In controlled ovarian hyperstimulation (COH) cycles, the aim is to develop numerous follicles to obtain an optimum number and quality of oocytes. Premature luteinizing hormone (LH) surge and premature ovulation during the treatment is one of the major causes of cycle cancellation (1). In gonadotropin releasing hormone (GnRH) antagonist protocol, GnRH antagonists (GnRHant) suppress endogenous gonadotropins in minutes. However, new cheaper stimulation regimens that do not compromise success rates are under investigation (2,3).

Studies have demonstrated that progesterone is an important regulator on timing of ovulation, and it can be used instead of GnRHant to prevent early luteinization. In the protocol, which is defined as progestin-primed ovarian stimulation (PPOS), progestins are used as an alternative to GnRHant to suppress LH surge. For this purpose, various progesterone forms and doses have been reported (4). Progesterone treatment controls oocyte development and timing of ovulation but, owing to disruption of synchronization between embryo development and endometrial receptivity due to untimely progesterone exposure, embryos obtained from these oocytes cannot be transferred in the same cycle (5). Nevertheless, improvements in cryopreservation and vitrification techniques enable the freezing of all oocytes or embryos to be transferred in subsequent cycles (2,6).

A few studies have shown that the application of this protocol does not adversely affect oocyte development and the number of oocytes obtained, compared to antagonist protocol (1,7,8). Besides, a flexible PPOS protocol has been defined recently (9).

The aim of this study was to investigate whether a synthetic progesterone, dydrogesterone, when used in a flexible protocol, is as effective as GnRHant in suppressing the LH surge. The effect of dydrogesterone on other cycle parameters was also investigated.

Material and Methods

This retrospective, case-control study was conducted in an infertility unit of a tertiary referral university hospital, involving patients aged between 23 and 41 years, for whom GnRHant or oral dydrogesterone was used to block premature LH surge in COH cycles between July 2018 and July 2019. Patients who applied for fertility preservation due to advanced age or malignancy, or who underwent controlled ovarian stimulation due to diminished ovarian reserve, unexplained infertility, endometriosis or male factor were included in the study.

In dydrogesterone group, starting from the third day of the cycle, 150-225 IU gonadotropin, either human menopausal gonadotropin (hMG) (Merional® 75IU, IBSA Institut) or recombinant follicle stimulating hormone (rFSH) (Gonal-f® 75IU, Merck-Serono) was administered. Dydrogesterone (Duphaston® 10 mg, Abbott Farma, Netherlands) 2x20 mg/day was started when the dominant follicle reached 12 mm in diameter or serum estradiol was over 300 pg/mL. Gonadotropin dose was adjusted according to the response of the ovary five days later and it was used until trigger day, while dydrogesterone was continued at a dose of 2x20 mg until trigger day. When two or more follicles reached 18 mm diameter, final oocyte maturation was triggered with 250 µg choriogonadotropin alpha (Ovitrelle® 250 mcg, Merck-Serono) and GnRHant (Decapeptyl® 0.1 mg, Ipsen Pharma or Lucrin® 5 mg/mL, Abbott). Regular and flexible PPOS protocols are shown in Figure 1a, b.

The control group consisted of age-matched patients, who received the GnRHant, cetrorelix (Cetrotide® 0.25 mg, Merck-Serono) by antagonist protocol. This group was given 150-225 IU gonadotropin, either hMG or rFSH, as detailed above. When the dominant follicle reached 13 mm in diameter, 0.25 mg cetrorelix was started and continued until trigger day.

Gonadotropin dose was also adjusted according to ovarian response and it was continued until trigger day. When two or more follicles reached 18 mm in diameter, final oocyte

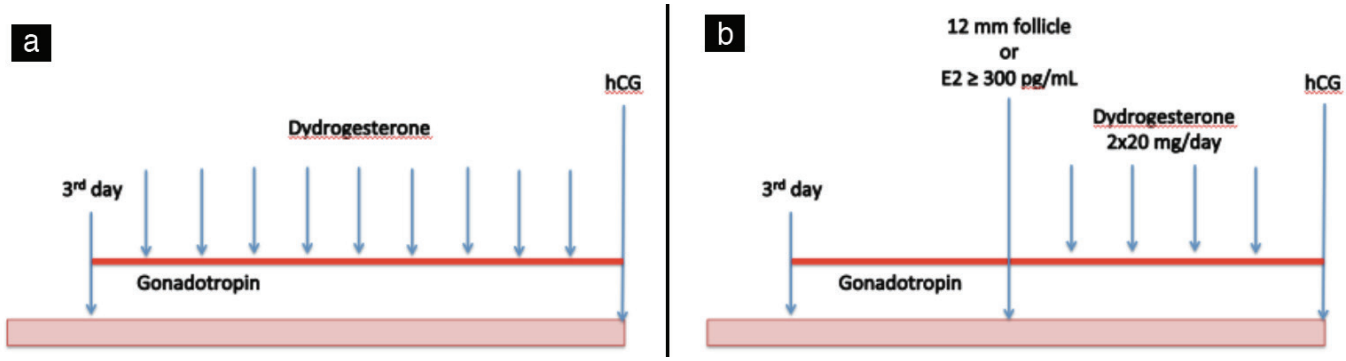

Figure 1. (a) Regular PPOS protocol, (b) Flexible PPOS protocol

PPOs: Progestin-primed ovarian stimulation

maturation was triggered with 250 μg choriogonadotropin alpha (Ovitrelle® 250 mcg, Merck-Serono) and GnRHant (Decapeptyl® 0.1 mg, Ipsen Pharma or Lucrin® 5 mg/mL, Abbott). In both groups oocyte retrieval was performed 36 hours after trigger application. Cryopreservation of the oocytes was performed using a vitrification technique.

Patients' records were examined and data items including age, body mass index, cause of infertility, type of COH protocol, duration and total dose of gonadotropins, duration of antagonist (cetrorelix)/dydrogesterone use, basal hormone levels, the suppression of premature LH surge, premature ovulation, and total and mature oocyte counts were collected and evaluated. Premature LH surge was defined as serum LH level >15 mIU/mL on trigger day (8). Premature ovulation was defined as rupture of the dominant follicle before oocyte retrieval and elevation of serum progesterone >3 ng/mL (8). Efficacy in suppressing premature LH surge was compared between oral dydrogesterone and GnRH antagonist.

A subgroup analysis of poor responder patients in both the study and control groups was further performed. This subgroup included patients belonging to group 3 and 4 according to Poseidon classification, that is patients with anti-mullerian hormone (AMH) <1.2 ng/mL or antral follicle count <5 (10).

This study was approved by Institutional Review Board and Ethics Committee of Ankara University Faculty of Medicine (approval number: 20-1364-18). This study was performed in accordance with Declaration of Helsinki. All patients gave informed consent prior to their treatment.

Statistical analyses

Data analyses were performed using SPSS, version 21.0 (IBM Corporation, Armonk, NY, USA). The variables were investigated using visual (histograms and probability plots) and analytical methods (Shapiro-Wilk test) to determine whether or not they were normally distributed. As data was non-normally distributed, non-parametric tests were used. Descriptive statistics of continuous variables were compared between groups using Mann-Whitney U test. The chi-square test or Fisher's exact test (when chi-square test assumptions do not hold due to low expected cell counts) were used to compare categorical variables between groups. Continuous variables are presented as median and interquartile range limiting the reported range to values between the 25-75th percentiles, whereas categorical variables are presented as number and percentage. A $p<0.05$ was considered statistically significant.

Results

In total 105 women participated in the study, of whom 52 were included in the dydrogesterone group and 53 in the GnRHant group. Demographic features, total duration and dose of gonadotropins, number of total oocytes and metaphase II oocytes collected were similar between the two groups (Table 1). However, total duration of dydrogesterone/cetrorelix administration was found to be significantly different ($p<0.001$). Trigger day estradiol was lower, while trigger day progesterone and maximum LH levels were higher in the dydrogesterone group. It was also remarkable that AMH levels were similarly low in both groups [0.80 (0.38-2.31) and 0.42 (0.30-4.0) ng/mL in the dydrogesterone and antagonist groups, respectively ($p=0.188$)].

Indications for COH were fertility preservation due to advanced age in 28 (26.7%) patients, fertility preservation due to malignancy in 12 (11.4%) patients, diminished ovarian reserve in 29 (27.6%) patients, unexplained infertility in

Table 1. Demographic and clinical features of the groups

	Dydrogesterone (n=52)	Cetrorelix (n=53)	p
Median (IQR) age (years)	33 (25-38)	32 (30-36)	0.527
Median (IQR) BMI	24.1 (19.7-25.4)	25.8 (23.3-28.6)	0.103
Median (IQR) parity	0 (0-0)	0 (0-0)	0.476
Median (IQR) AMH (ng/mL)	0.80 (0.38-2.31)	0.42 (0.30-4.0)	0.188
Median (IQR) duration of pituitary suppression (days)	6 (4-7)	4 (3-6)	**<0.001**
Median (IQR) duration of gonadotropins (days)	10 (8-11)	9 (8-11)	0.110
Median (IQR) total dose of gonadotropins (IU)	2025 (1800-2475)	1950 (1560-2712)	0.809
Median (IQR) trigger day E_2 (pg/mL)	748 (150-1060)	1395 (550-2382)	**0.007**
Median (IQR) trigger day P (ng/mL)	1.27 (1.0-2.29)	1 (0.80-7.75)	**0.004**
Median (IQR) maximum LH (mIU/mL)	6 (5.0-10.47)	4 (2.5-16)	**0.005**
Median (IQR) number of oocytes	8 (2-12)	8 (2-13)	0.669
Median (IQR) number of MII oocytes	6 (1-10)	7 (1-11)	0.399
Premature ovulation, n (%)	6 (11.5)	0 (0)	**0.013**

BMI: Body mass index, AMH: Anti-mullerian hormone, E_2: Estradiol, P: Progesterone, LH: Luteinizing hormone, IQR: Interquartile range, MII: Metaphase II

3 (2.9%) patients, endometriosis in 14 (13.3%) patients, and male factor in 19 (18.1%) patients (Table 2). There was no difference between the dydrogesterone and GnRHant groups in terms of indications (p=0.215).

Dual trigger was used in all patients, with the exception of five patients in whom estradiol was >4000 pg/mL on trigger day, when analog trigger was applied. The two groups were also similar with respect to application of trigger agents.

Premature LH surge was present in 13.5% in dydrogesterone group and in 9.4% of the antagonist group, which was not significant (p=0.517). However, premature ovulation before the scheduled oocyte pick up day occurred in 6 (11.5%) patients in dydrogesterone group, whereas it did not occur in antagonist group (p=0.013). In four of these six patients, oocytes could be collected from the other follicles, while oocyte retrieval failed in the remaining two patients due to diminished ovarian reserve.

The subgroup of poor responder patients included 40 patients, of whom 25 (62.5%) were in the dydrogesterone group and 15 (37.5%) were in the antagonist group. Clinical outcomes were similar poor responders in both groups but all six patients who had premature ovulation were in the dydrogesterone group (Table 3).

Discussion

This study demonstrated that dydrogesterone, used in a flexible PPOS protocol, provided similar results in some respects to antagonist protocol in preventing premature LH surge. Favorable results included total number and quality of oocytes collected. However, a high incidence of premature ovulation in patients receiving dydrogesterone suggested that flexible regimen might not be suitable in all patients.

Transient, but quick, LH suppression provided by GnRHant is associated with competitive blockade of GnRH receptors (8,11), while progestins suppress GnRH secretion at the hypothalamus when they are administered in the early phase of the cycle and prior to estrogen elevation (8,12). It has been stated that serum LH levels are more stable with PPOS and oocyte retrieval can be planned more precisely (8). Furthermore, ease of oral use instead of daily injections and lower cost of treatment are advantageous (7,9,13). However, as the main limitation of these protocols is the inability to perform transfer in the same cycle, progestins may be more suitable for planned freeze-all cycles, pre-implantation genetic testing cycles, elective oocyte cryopreservation and oocyte donor stimulation (14).

Table 2. Indications of controlled ovarian hyperstimulation

	Dydrogesterone (n, %)	Cetrorelix (n, %)	Total number of patients
Fertility preservation due to advanced age	12 (42.9%)	16 (57.1%)	28
Fertility preservation due to malignancy	9 (75.0%)	3 (25.0%)	12
Diminished ovarian reserve	16 (55.2%)	13 (44.8%)	29
Unexplained infertility	1 (33.3%)	2 (66.7%)	3
Endometriosis	8 (57.1%)	6 (42.9%)	14
Male factor	6 (31.6%)	13 (68.4%)	19

Table 3. Demographic and clinical features of the patients in the poor responder subgroup

	Dydrogesterone (n=25) (48.1%)	Cetrorelix (n=15) (28.3%)	p
Median (IQR) age (years)	32 (25-38)	35 (32-37)	0.595
Median (IQR) BMI	24.7 (19.6-30.1)	26.6 (23.4-29.9)	0.199
Median (IQR) parity	0 (0-0)	0 (0-0)	0.289
Median (IQR) AMH (ng/mL)	0.34 (0.09-0.74)	0.42 (0.22-0.58)	0.903
Median (IQR) duration of pituitary suppression (days)	5 (4-6)	4 (3-6)	0.397
Median (IQR) duration of gonadotropins (days)	8 (6-9)	10 (8-12)	0.820
Median (IQR) total dose of gonadotropins (IU)	1800 (1425-2025)	2213 (1631-3600)	0.345
Median (IQR) trigger day E_2 (pg/mL)	157 (78-724)	408 (335-1132)	0.108
Median (IQR) trigger day P (ng/mL)	1.0 (0.6-1.5)	5.0 (1.0-9.5)	0.054
Median (IQR) maximum LH (mIU/mL)	9.0 (6.3-14.3)	11.5 (7.5-17.0)	0.897
Median (IQR) number of oocytes	2.0 (0.5-5.0)	2.0 (1.0-3.3)	0.283
Median (IQR) number of MII oocytes	2.0 (0.5-4.0)	1.5 (1.0-2.3)	0.377
Premature ovulation, n (%)	6 (24)	0 (0)	**0.046**

BMI: Body mass index, AMH: Anti-mullerian hormone, E_2: Estradiol, P: Progesterone, LH: Luteinizing hormone, MII: Metaphase II

Progesterone is known to inhibit estradiol-induced LH surge, both in early follicular phase and early luteal phase (1). Although there is much concerning endogenous LH surge and the role of progesterone to be elucidated (15), it has been reported that progesterone should be administered at the right time to be effective (1). Recently, multiple follicle selection waves and random start protocols have brought attention to flexible PPOS programs, and in a study, including donor cycles, it was shown that a flexible PPOS protocol can effectively suppress premature ovulation as well (9).

In our study, cycle parameters other than the incidence of early ovulation were mostly similar between the groups. Notably, total duration of pituitary suppression was longer in dydrogesterone group, since we started to use dydrogesterone one day earlier than is normal with GnRHant administration protocols and this result is similar to some previous studies. In the report of Kuang et al. (1), while hMG dose and duration were higher in the study group, collected oocyte counts and other cycle parameters were similar. In the study of Xiao et al. (7), while dose and duration of gonadotropins were higher in the PPOS group, other characteristics were similar. Cycle parameters were similar in the study of Chen et al. (8), and were also similar in the study of Wang et al. (16), with the exception of hMG dose being higher in the PPOS group. Lower trigger day estradiol levels in the dydrogesterone group in our study were probably caused by a higher number of poor responder patients (25/52) in this group. Also, the difference between LH levels, which were <10 mIU/mL in both groups, was not considered clinically significant, the higher trigger day progesterone levels in our dydrogesterone group were consistent with the finding of premature ovulation.

Patients who underwent COH for a range of indications were included in our study. Data regarding different patient groups using PPOS protocols are available in the literature. PPOS is reported to be successful in polycystic ovary syndrome (PCOS) patients as an alternative to the antagonist protocol, as it reduces the risk of ovarian hyperstimulation syndrome, suppresses premature LH surge, and uses a freeze-all strategy (7,16). It has been suggested that higher total gonadotropin dose and duration with PPOS are caused by decreased follicle sensitivity due to high progesterone and pituitary suppression (7). On the other hand, there are also studies which report that PPOS suppresses LH surge better than GnRHant, with similar oocyte counts, in poor responder patients (8,17,18). Among our patients who received treatment for fertility preservation due to malignancy, PPOS protocol was not administered to patients with breast cancer, while two of the patients in the cetrorelix group had breast cancer. These two patients received letrozole 5 mg/day (Femara®, Novartis, Switzerland), starting from the third day of the menstrual cycle along with gonadotropin, until trigger day.

Synthetic progesterones are preferred in studies as natural micronized progesterones can affect serum values, and medroxyprogesterone acetate (MPA) is the most commonly used agent for this purpose. Kuang et al. (1) compared 10 mg/day MPA with standard antagonist protocol for the first time, and found similar results in terms of hMG dose and duration, as well as oocyte and embryo counts. It was previously reported that MPA could not inhibit ovulation at a dose of 5 mg/day (19). However, Dong et al. (20), who investigated the minimum dose to suppress LH surge, concluded that 4 mg MPA was similar to 10 mg in terms of the number of oocytes collected and was sufficient to prevent premature LH surge. Yu et al. (4) compared MPA and dydrogesterone to suppress premature LH surge, and reported that premature LH surge was not seen in either group, and similar oocyte counts and cycle characteristics were observed between the groups. Nevertheless, there are few studies using dydrogesterone, and no exact protocol in terms of dose and duration has been specified. There are reports that dydrogesterone does not prevent ovulation at recommended doses (10-20 mg/day) for MPA, and a minimum dose of 30 mg dydrogesterone is required for this purpose (21,22). Yu et al. (4) also concluded that dydrogesterone is less proficient than MPA in suppressing GnRH, and a minimum 20 mg dose is needed to be effective. Based on these reports, while 30 mg/day is considered to be a suitable dose for dydrogesterone, in order to provide patient compliance, 40 mg/day (2x2 tablets) dydrogesterone was preferred in our study.

Rates of premature LH surge were 13.5% and 9.4% in dydrogesterone and cetrorelix groups respectively, which was not significantly different. In the study of Kuang et al. (1), premature LH surge was not observed in either group, and only occurred in 1 in 150 of the whole cohort. In the study of Chen et al. (8) in the poor responder group, the rate of premature LH surge was significantly lower in the PPOS group compared to the antagonist group. However, the number of obtained oocytes and embryos were similar. In the study of Wang et al. (16) in the PCOS group, premature LH surge and premature ovulation were not reported, and the cycle parameters were similar, with the exception of higher hMG doses in the MPA group. However, there are also studies reporting higher rates of premature LH surge. Although the suppressive effect of GnRHant on LH is rapid and reversible, premature LH surge is reported but is extremely variable at 0.34-38% of the patients (23,24). When compared to the prompt effect of GnRHant, dydrogesterone acts more slowly as its peak plasma level is achieved after one hour (21).

Collection of oocytes before ovulation in some of our patients with premature LH surge was accomplished by changing the oocyte aspiration time, based on LH monitoring. This positive effect of LH monitoring has also been reported in previous

studies. Chen et al. (8), in their study with poor responders, observed that at least one mature oocyte could be collected from 9 of 10 patients who had premature LH surge in the antagonist group by changing the oocyte retrieval time. LH monitoring was not performed in the flexible PPOS protocol study of Yildiz et al. (9) but premature ovulation was not reported for either group, though this study included donor cycles and not poor responders.

In our study, dual trigger was used for completion of final maturation, in line with the results of previous studies (1,16,25). While premature ovulation was not observed in our cetrorelix group, this was observed in the dydrogesterone group, which was a significant difference. It was formerly reported that, rise in serum progesterone level after the increase in serum estradiol concentrations results in earlier LH surge (26,27). Therefore, higher premature LH surge and premature ovulation rates in our dydrogesterone group may be related to the late administration of dydrogesterone in our study, when compared to these earlier studies, in which progesterone administration was started at the third day of the cycle (1,4,8). It is also reported that diminished ovarian reserve increases the risk of premature LH surge in antagonist cycles (24). Although we classified our cohort due to COH indications, 48.1% and 28.3% of the patients were poor responders in dydrogesterone and GnRH antagonist groups respectively. It has been demonstrated that follicular phase may be shortened in older ovulatory women due to earlier dominant follicle selection. Moreover, low response may also be associated with accelerated luteinization of mature follicles (28,29). The relatively high prevalence of poor responder patients may be a potential reason of advanced follicular maturation, and possibly premature ovulation. Therefore, particularly in patients with diminished ovarian reserve, flexible protocol may not be suitable, and early administration of dydrogesterone may be necessary to prevent premature ovulation. However, Turkgeldi et al. (18) recently reported that flexible PPOS protocol might be used as an alternative to the flexible GnRHant protocol in patients with diminished ovarian reserve. In this study, pituitary suppression by MPA was commenced as the estradiol level was ≥200 ng/L in contrast to the 300 ng/L threshold of estradiol in our study. A similar threshold level was used in the present study with our normal flexible GnRHant protocol since there is no clear cut-off for flexible PPOS protocols, and only one premature ovulation was encountered in the study group, which consisted of 27 patients. These findings suggest that dydrogesterone should be administered earlier but further research is required to confirm this suggestion.

In patients with premature ovulation, oocytes could be retrieved from 4 of 6 of them. It was also reported in previous studies that fertilization and live birth could be achieved from the oocytes of the smaller follicles as well as the oocytes collected from cul-de-sac after premature ovulation (30).

Study limitation

The main limitation of this study is its retrospective nature. However, a lack of difference between the demographic characteristics of both groups may decrease the risk of bias that may occur. Besides, while live birth rate is an important parameter in evaluating cycle success, pregnancy outcomes could not be assessed due to freeze-all strategy, and in particular, cryopreservation of the oocytes for fertility preservation in a not inconsiderable proportion of the patients.

Conclusion

Dydrogesterone can be used as an alternative to antagonist regimen in patients, where embryo transfer is not planned for the same cycle. However, particularly in patients with diminished ovarian reserve, early initiation of progesterone may be appropriate, owing to advanced and accelerated follicular maturation and due to the oral absorbtion pharmacokinetics of dydrogesterone. However, randomized controlled trials in particular populations are required to determine the most effective minimum dose and time of application to ensure treatment success.

Ethics Committee Approval: *This study was approved by Institutional Review Board and Ethics Committee of Ankara University Faculty of Medicine (*approval number*: 20-1364-18).*

Informed Consent: *All patients gave informed consent prior to their treatment.*

Peer-review: *Externally peer-reviewed.*

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Reference ranges for flow velocities and the indices of the ductus venosus in low-risk pregnancies

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Abstract

Objective: Ductus venosus blood flow velocity measurements are mandatory in many clinical indications. The evaluation of the flow is performed either by comparing results with general reference tables or by qualitative assessment of the "a" flow, in regard to reversed or absent flow in the spectral waveforms. The aim was to develop normal reference ranges in low-risk pregnancies in our population.

Material and Methods: Measurements of flow velocities (S, v, D, a) and indices (pulsatility index for veins, peak velocity index for veins, a/S, S/a) were performed by a single experienced specialist in 1279 singleton, uncomplicated pregnancies between 11 and 40 weeks gestation. The absolute flow velocities (S, v, D, a, VmPeak) and indices were obtained from spectral waveforms using the equipment producer's inbuilt system. The still images were stored in the picture archiving and communication system.

Results: The predicted reference ranges of the ductus venosus blood flow velocities according to the gestational age are shown in tables and graphics. Predicted reference curves based on the 5th and 95th percentiles according to gestational week were plotted and are given in tables and figures.

Conclusion: Normal reference ranges for absolute flow velocities and indices were calculated from a population of uncomplicated pregnancies attending a tertiary care center. The measurements were made from both the classic patterns of the waveforms and also considered variants of the spectral waveforms, which have recently been reported, for the first time in the medical literature. (J Turk Ger Gynecol Assoc 2021; 22: 300-11)

Keywords: Doppler, ductus venosus, ultrasonography

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Introduction

Ductus venosus (DV) Doppler assessments play a critical role, not only for the diagnosis of chromosomal abnormalities and congenital cardiac defects in early pregnancy, but also for determining cardiovascular health in the follow up of the pregnancy. In the UK DV Doppler assessment has been suggested for use in surveillance and timing of delivery (1).

The DV spectral waveform pattern has two peaks (S & D) and two nadirs (v & a) in a cardiac cycle. The "S" and "D" peaks correspond to the maximum and "v" and "a" nadirs correspond to the minimum intra-atrial pressure, which accelerates or decelerates the forward flow in DV throughout a cardiac cycle (Figure 1-7, Video 1-3). The flow in DV is assessed by either qualitatively observing the spectral pulsed Doppler (PD) flow and checking the "a" nadir in the waveform to assess if it is "reversed" or "not", and or quantitatively comparing the measurements with published reference ranges for each gestational week of pregnancy (2-5).

Even though there are already published reference values, advancing technologies to visualize the DV and obtaining reproducible measurements are now easier. Therefore reassessment and publication of reference limits is warranted. The aim of this study was to derive reference ranges of sonography angle-dependent absolute flow velocities for S, v, D, a and VmPeak and angle-independent Doppler indices of

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pulsatility index for veins (PIV), peak velocity index for veins (PVIV), a/S, S/a, preload index [(S - a)/S)] (6,7) and SIA index [S/(v + a)] (8) (Figure 1).

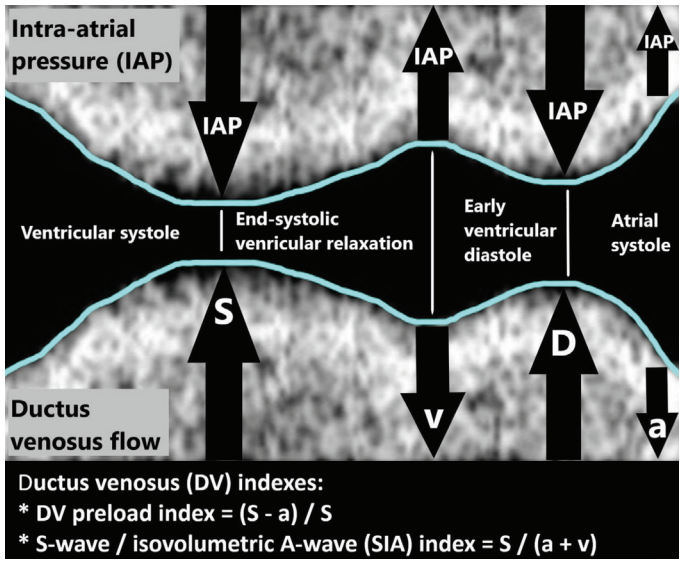

Figure 1. Relationships between the DV flow velocities and the intra-atrial pressure changes in a cardiac cycle. DV flow velocity increased while intra-atrial pressure decreased and *vice versa*

S: Velocity in ventricular contraction, v: Velocity in end-systolic ventricular relaxation, D: Velocity in early ventricular diastole, a: Velocity in atrial systole

Figure 2. The measurement of ductus venosus flow velocity and indices 12 weeks' gestation

DR: Dynamic range, DF: Dynamic frequency, CG: Color gain, F: filter, DV: Ductus venosus, S: Velocity in ventricular contraction, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

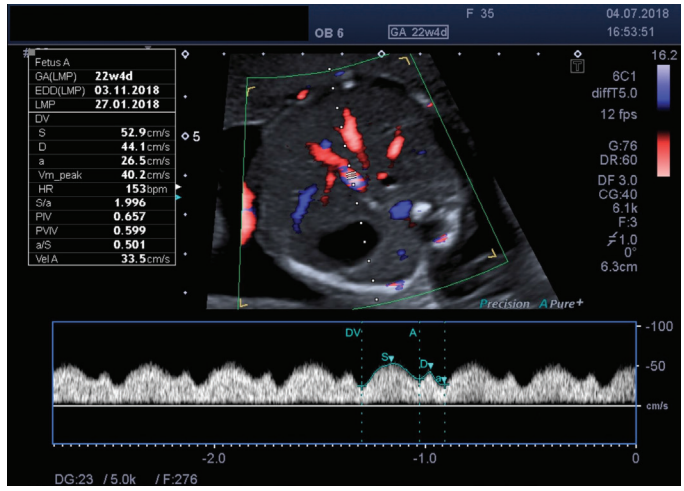

Figure 3. The measurement of ductus venosus flow velocity and indices 22 weeks' gestation in the oblique transverse plane

DR: Dynamic range, DF: Dynamic frequency, CG: Color gain, F: filter, DV: Ductus venosus, S: Velocity in ventricular contraction, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

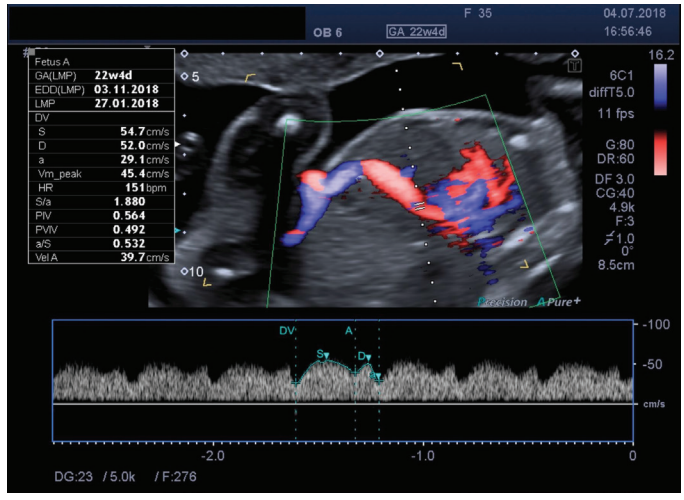

Figure 4. The measurement of ductus venosus flow velocity and indices 22 weeks' gestation of the same patients in figure 3 in the sagittal plane

DR: Dynamic range, DF: Dynamic frequency, CG: Color gain, F: filter, DV: Ductus venosus, S: Velocity in ventricular contraction, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

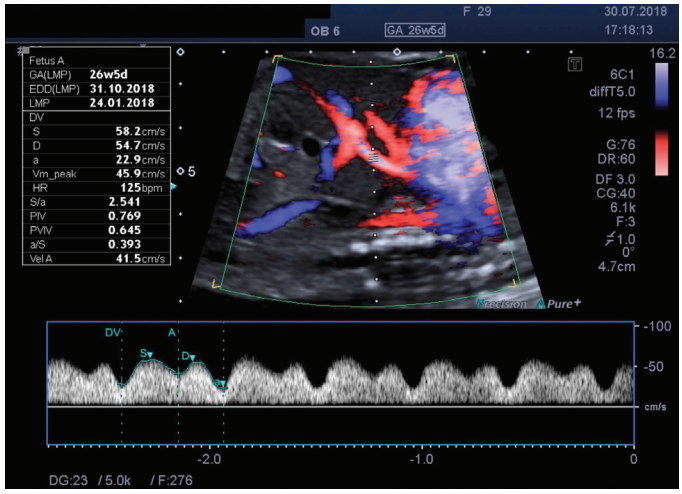

Figure 5. The measurement of ductus venosus flow velocity and indices 26 weeks' gestation

DR: Dynamic range, DF: Dynamic frequency, CG: Color gain, F: filter, DV: Ductus venosus, S: Velocity in ventricular contraction, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

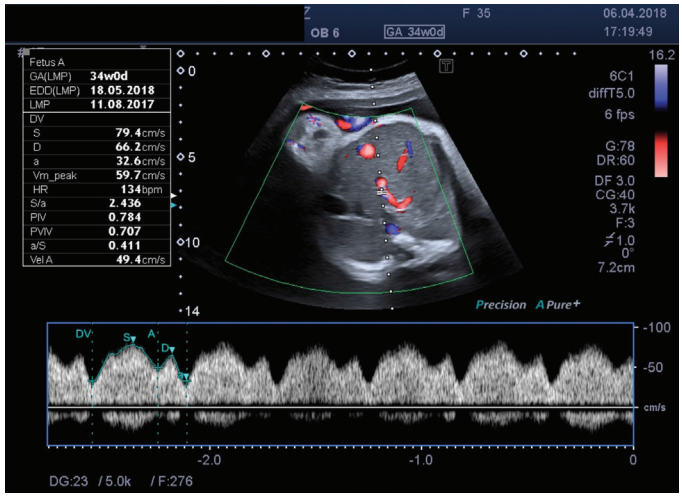

Figure 6. The measurement of ductus venosus flow velocity and indices 34 weeks' gestation in the oblique transverse plane

DR: Dynamic range, DF: Dynamic frequency, CG: Color gain, F: filter, DV: Ductus venosus, S: Velocity in ventricular contraction, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

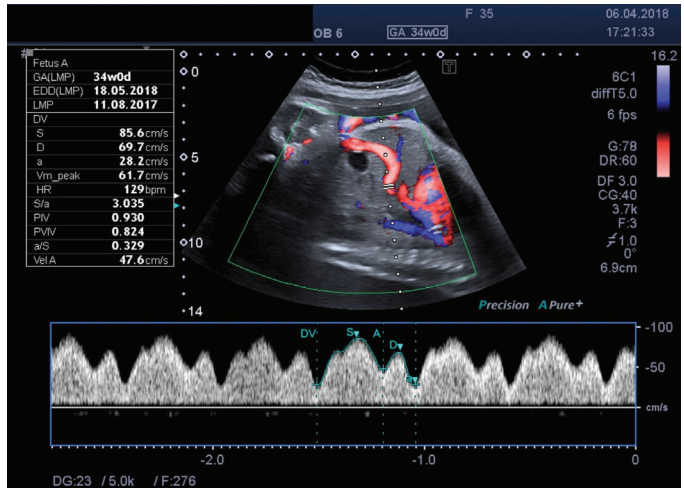

Figure 7. The measurement of ductus venosus flow velocity and indices 34 weeks' gestation of the same patients in figure 6 in the sagittal plane

DR: Dynamic range, DF: Dynamic frequency, CG: Color gain, F: filter, DV: Ductus venosus, S: Velocity in ventricular contraction, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

Material and Methods

Singleton, low-risk pregnancies fulfilling the inclusion criteria between 11 and 40 weeks gestation were included in this prospective study that took place between January 2016 and February 2018. Inclusion criteria were selected to ensure that the pregnancy was either uncomplicated or that there were no risk factors for maternal or fetal safety. Since the hospital is a tertiary care center, the patients were either from the urban or the rural territories of the city, which takes immigration from several parts of the country.

The written informed consent from the patients and the ethical approval from the Ethics Committee of the University of Health Sciences Turkey, Antalya Training and Research Hospital were obtained (approval number: 16/13, date: 02.11.2017).

Inclusion criteria were: (1) pregnancies with optimal visualization of the DV using a wideband color Doppler technique [advanced dynamic flow (ADF)]; (2) eventual Apgar score ≥8; (3) birth weight ≥2500 grams; (4) measurements taken from a single optimal waveform after obtaining 4 to 5 consecutive uniform Doppler velocity waveforms in the tracings with 2-3 cm/s sweep speed; (5) patients with normal amniotic fluid index; and (6) patients with normal screening tests.

Exclusion criteria were: (1) gestational diabetes; (2) multiple pregnancies; (3) preeclampsia; (4) maternal cardiac rhythm

disturbances; (5) maternal smoking during pregnancy; (6) polyhydramnios or oligohydramnios; (7) fetal morphological abnormality; (8) intrauterine growth restriction; (9) macrosomia; (10) fetal stillbirth; (11) eventual Apgar score <8; and (12) fetuses with transient bradycardia (deceleration).

In all cases the equipment used for all examinations was a Toshiba Applio 500 system (TUS-A500, Toshiba Medical Systems Europe B.V., Zilverstraat 1, 2718 RP, Zoetermeer, the Netherlands) with a 2-6 MHz broadband convex transducer.

Measurements of DV flow velocities (S, v, D, a, VmPeak) and indices (PIV, PVIV, a/S, S/a) were performed using a spectral waveform. Isovolumetric relaxation velocity (IRV or DV v) was measured manually, since it was not inbuilt into the equipment capabilities. The preload and SIA indices were calculated using Microsoft Excel (Microsoft Corp., Santa Rosa, CA, USA). The still images were stored in the Sectra picture archiving and communication system (PACS) system (Sectra AB, Teknikringen 20, SE-583 30 Linköping, Sweden).

DV measurements were performed in all variants of the spectral waveform patterns of the DV flow, which were described recently by Gürses et al (9). Calculations were made either in the normal component of type 4 or type 5 flow patterns, or after the flow pattern returned to a classic pattern either in type 6 or 7 flow patterns.

Fetal age was estimated according to the last menstrual period. However, in case of discordance of >7 days between the age based on the last menstrual date and the age based on the biometry measures with ultrasonography, the gestational age was redated.

Doppler examinations are performed by a single experienced specialist (C.G.), certified for DV flow and Doppler examinations by the (Fetal Medicine Foundation ID: 127129; 137 Harley Street, London, W1G 6BG, United Kingdom). DV measurements were performed according to the criteria in the medical literature (10-13). A 1 mm wide sample gate was positioned over the isthmus and adjacent proximal section, where the aliasing occurs due to the accelerated jet flow. The wide-band color Doppler technique (ADF) was used for mapping the DV and placing the sample gate accurately in PD examinations to avoid contamination of the signal by adjacent veins during the PD tracings (Figure 2-7, Video 1-3).

The still images were stored in the Sectra (PACS) system (Sectra AB, Teknikringen 20, SE-583 30 Linköping, Sweden).

Toshiba Applio 500 system (TUS-A500, Toshiba Medical Systems Europe B.V., Zilverstraat 1, 2718 RP, Zoetermeer, the Netherlands) is used in all examinations with a 2-6 MHz broadband convex transducer.

The acoustic output level was adjusted to a minimum following the as low as reasonably achievable principle and the maximum mechanical index was 1.1. Imaging parameters were dynamic range 70, dynamic frequency 3.0, and color gain 40, color pulse repetition frequency 5-6 and color filter (F) 3-4. Sweep speed was 2-3 cm/s in all of the trimesters and the Doppler filter was set between 90 and 140 in the first trimester of the pregnancy during the PD examinations.

Statistical analysis

Data were collected using Microsoft Excel for Windows (Microsoft, Redmond, WA, USA) and the analysis was performed using the Statistical Software for Social Sciences for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation. For statistical analysis, the distribution of each blood flow velocity and DV index was examined using scatter plots against the gestational age. Due to the descriptive nature of the study, no tests of statistical significance were performed.

Results

A total of 1279 singleton pregnancies between 11 and 40 weeks gestation were enrolled in this prospective, cross-sectional study. The mean maternal age was 28.05±6.54 years and the mean eventual birth weight was 3,294±448.5 g. Median (range) gestational age was 22 (11-40) weeks and mean body mass index was 29.05±5.17. The median (range) number of participants for each week of gestation was 35 (23-86).

Peak forward velocities were recorded and DV indices were calculated (Figure 1-7). The predicted reference ranges of the DV blood flow velocities and indices by gestational age are shown in Table 1-6. Predicted reference curves based on the 5th and 95th percentiles by gestational week were plotted and shown in Figure 8-10.

Discussion

After ultrasonographic velocimetry of fetal DV was described by Kiserud et al. (14) for the first time, there has been interest in establishing reference ranges for the measurements obtained. Several studies have published reference ranges for either the absolute flow velocities, which are insonation angle-dependent, or the indices, which are clinically preferred due to being the insonation angle-independent, for all weeks of pregnancies (2-5,15).

Doppler assessment of DV should satisfy some criteria, which have previously been discussed in detail (10-13). In the present study, these criteria were held constant in terms of user preset adjustment. In addition, wideband color Doppler technique was used to image the DV and to place the sample gate before performing measurements. The advantage of wideband color Doppler

is that blooming is thought to be less problematic than when using the conventional narrowband technique. Blooming is the name given to an artefact of color Doppler where vessels appear larger than the true diameter due to aretfactual extension of the color signal beyond the vessel walls. Blooming artefact is the main disadvantage associated with visualization of the vascular structures due to a lack of lateral discrimination and it causes exaggerated or false positive vascular colorizing (16,17). Blooming is particularly important when attempting to visualize the tiny or slow-flow blood vessels of the fetus. Blooming increases not only false vascularity but also wrong placement of the sample gate in PD tracings and causes prolonged examination time. Maiz et al. (10) demonstrated that a sonographer is required to perform an average of 80 examinations to achieve competence in the Doppler assessment of DV. One of the criteria of Maiz et al. (10) was to obtain measurements in a right ventral mid-sagittal view. However, it has been suggested that the main criterion should be to position the probe so that

Table 1. Predicted reference ranges for ductus venosus spectral Doppler absolute flow velocities from 11 to 20 weeks of gestation

Week	Percentile	S	v	D	a	VmPeak
11	5	20.7	0	16.5	5.58	15.5
	50	35.2	24.7	30.5	10	26.3
	95	46.4	36.9	42.6	23	37.8
12	5	23.9	0	21	6.2	17.6
	50	37.0	24.2	31.8	10.9	27.6
	95	51.3	40	47	20.3	39.4
13	5	24.8	0	22.8	4.9	18.2
	50	36.9	24.6	32.7	10.9	27.8
	95	50.6	38.7	45.6	20.8	39.3
14	5	30.3	0	27.7	5.4	23.2
	50	43.3	30.5	38.7	11.9	33.3
	95	58.3	41.9	50.4	18.4	43.5
15	5	24.9	0	20.2	6.7	17.4
	50	46.5	34.6	41.7	15.2	36.3
	95	61.6	50.9	56.8	27.2	50.5
16	5	29.4	19.6	26	9.7	22.9
	50	52.6	39.7	47.2	17.9	41.4
	95	60.9	51.2	56.7	32.1	50.6
17	5	31.8	13.2	29.9	10	26.1
	50	45.6	35.2	40.7	17.6	36.9
	95	64.3	49.4	57.1	31.7	50.2
18	5	34.9	18.6	32.6	10.9	27.6
	50	52.9	41.8	50.3	21.8	43.5
	95	66.9	53.1	61	35.7	54.6
19	5	32.3	24.3	29.6	11.4	25.4
	50	50.1	39.7	47.1	21.5	41.3
	95	68.6	54.3	62	40	56.3
20	5	30.1	5	28.1	11.2	25.1
	50	49.4	39	46.7	24.8	40.3
	95	74.4	57.3	70.2	40.3	61.6

S: Velocity in ventricular contraction, v: Velocity in end-systolic ventricular relaxation, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity

Table 2. Predicted reference ranges for ductus venosus spectral Doppler absolute flow velocities from 21 to 30 weeks of gestation

Week	Percentile	S	v	D	a	VmPeak
21	5	38.8	16.9	33.9	13.3	29.9
	50	57.6	42.7	52.6	27.5	46.4
	95	73	56.6	67.9	42.6	60.5
22	5	39.7	22.1	35.4	15.9	31.1
	50	55.1	42.7	51.4	26.6	45.5
	95	73.7	61.2	66.1	46.6	61.1
23	5	39.4	28.8	35.6	16.9	31.2
	50	62	45	55.1	30.4	48.9
	95	80	62.1	72.8	47.6	67.4
24	5	56.3	0	49.3	25.6	45.5
	50	64.5	50.6	61.5	34.4	53.5
	95	81.5	63.3	73.4	50.6	66.6
25	5	39.2	18.7	34.2	14.5	27.2
	50	58.5	44.1	56.5	31.8	49.7
	95	81.4	64.7	74.5	53.8	67.9
26	5	42.1	25.6	37.3	18.2	32.4
	50	58.2	43.2	52.9	30.9	46.6
	95	77.3	59.7	72.7	49.2	64.6
27	5	39	26.1	33.5	15.9	30.5
	50	58.2	44.1	52.6	27.3	47.6
	95	85.9	71	78.9	54.2	74.6
28	5	31.3	20.4	28	13.9	23.8
	50	62.6	48.6	59.1	37.6	53.5
	95	76.9	62.5	72.4	50.6	65.8
29	5	41.7	28	35.7	19.1	33.4
	50	62.6	48.5	57.6	38.7	52
	95	79.4	66.7	74.1	51.3	68.9
30	5	39.5	24.7	35.3	14.5	31.6
	50	58.5	43.6	54.2	32.6	48
	95	92	77.1	88.2	55.9	79.5

S: Velocity in ventricular contraction, v: Velocity in end-systolic ventricular relaxation, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity

the blood flow direction is towards the transducer plane which will result in an insonation angle close to 0 (Figure 1, Video 1). The wideband color Doppler technique allows visualization of the intrahepatic umbilical vein (UV), the left portal vein (LPV) and the portal sinus (PS) not only in ventral but also in dorsal approach (Figure 1, Video 1).

Even though Martins and Kiserud (13) suggested using a sample gate setting of 2-5 mm wide in the second half of the pregnancy, in the present study a 1 mm wide sample gate was used consistently and for measurements made at each gestational week to allow direct comparison with measurements made in other gestational weeks and to avoid signal contamination from the hepatic veins and inferior vena cava.

Despite the difficulty of attempting to ensure an insonation angle of the vessel of 0 degrees, the absolute flow velocities

Table 3. Predicted reference ranges for ductus venosus spectral Doppler absolute flow velocities from 31 to 40 weeks of gestation

Week	Percentile	S	v	D	a	VmPeak
31	5	37.4	26.2	35.7	21.8	31.9
	50	57.8	44.5	53.8	32.2	49.5
	95	77.9	65.4	72.6	49.9	66.2
32	5	30.2	25.7	28.2	14.6	25.9
	50	57.4	43.2	51.6	30.2	47.9
	95	82.2	64.3	76.3	48.9	68.8
33	5	40.3	32	37.4	20	35.5
	50	57.9	46.6	52.5	33.9	49.0
	95	89.4	72	84.4	57.7	76.8
34	5	30.4	24.8	28.3	16.8	26.8
	50	59.2	41.5	53.2	30.1	47.7
	95	82.6	58.8	73.8	49.9	65.2
35	5	30.9	19.9	25.7	15	25.6
	50	53.6	40.7	50.6	31.6	46.3
	95	86	65.3	78.2	56.4	71
36	5	32.6	27.6	32.3	18.5	29.8
	50	54	42	49.5	31.3	44.6
	95	80.3	65	75	52.2	68.1
37	5	29.6	26.1	28	19.1	26.3
	50	57.6	43.1	52	34.4	46.9
	95	79.9	70.6	74.7	55.5	62.4
38	5	32.8	23.2	29.7	14.3	26.3
	50	52.9	44.1	49.4	31.9	46.2
	95	86.4	65.4	73.3	51.3	67.3
39	5	29.9	23.9	27.1	15.7	24.7
	50	49.9	40.6	46.7	31.8	41.8
	95	74	61	66.6	47.3	61.9
40	5	32	22.7	27.8	16.4	26.5
	50	43.1	37	39.7	31.6	39.7
	95	72.1	57.2	64.2	44.9	59.1

S: Velocity in ventricular contraction, v: Velocity in end-systolic ventricular relaxation, D: Velocity in early ventricular diastole, a: Velocity in atrial systole, VmPeak: Time-averaged maximum velocity

Table 4. Predicted reference ranges for ductus venosus Doppler indices from 11 to 20 weeks of gestation

Week	Percentile	PIV	PVIV	a/S	S/a	Preolad index	SIA index
11	5	0.52	0.43	0.17	1.77	0.43	0.69
	50	0.93	0.80	0.29	3.41	0.71	0.94
	95	1.13	1.03	0.56	5.62	0.82	1.12
12	5	0.62	0.55	0.18	2.05	0.51	0.75
	50	0.94	0.81	0.30	3.33	0.7	1
	95	1.15	1.01	0.48	5.43	0.81	1.25
13	5	0.59	0.51	0.15	1.93	0.48	0.77
	50	0.92	0.78	0.31	3.19	0.69	0.98
	95	1.14	0.99	0.51	6.34	0.84	1.31
14	5	0.69	0.61	0.13	2.24	0.55	0.81
	50	0.95	0.83	0.27	3.65	0.73	1
	95	1.2	1.03	0.44	7.34	0.86	1.24
15	5	0.61	0.53	0.22	2.01	0.5	0.75
	50	0.87	0.73	0.33	2.98	0.66	0.93
	95	1.05	0.90	0.49	4.50	0.78	1.17
16	5	0.51	0.45	0.21	1.77	0.43	0.72
	50	0.75	0.65	0.39	2.55	0.61	0.88
	95	1.04	0.92	0.56	4.70	0.79	1.09
17	5	0.4	0.36	0.26	1.57	0.36	0.65
	50	0.74	0.66	0.4	2.46	0.59	0.85
	95	1.04	0.87	0.63	3.73	0.73	1.1
18	5	0.38	0.34	0.25	1.52	0.34	0.64
	50	0.68	0.61	0.44	2.26	0.56	0.83
	95	1.02	0.84	0.65	4.04	0.74	1.12
19	5	0.41	0.36	0.32	1.56	0.36	0.66
	50	0.71	0.62	0.41	2.42	0.58	0.82
	95	0.87	0.74	0.63	3.08	0.67	0.95
20	5	0.34	0.32	0.3	1.43	0.30	0.67
	50	0.62	0.56	0.48	2.07	0.52	0.79
	95	0.96	0.82	0.69	3.31	0.69	1.09

PIV: Pulsatility index for veins, PVIV: Peak velocity index for veins, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, Preload index: (S-a)/S, SIA index: S/(a+v), Atrial preload index: (S+D)/(a+v), Atrial afterload index: (a+v)/(S+D)

were measured in DV at 0 or close to 0 degrees in most cases and certainly below 30 degree of insonation in the present study. Wideband color Doppler was also useful in determining the actual blood flow direction, so thereby deciding the angle of insonation. DV absolute flow velocities are increased in anaemia and diseases affecting the liver parenchyma (18,19), and the angle of insonation is critical for absolute flow velocity measurements in DV. In the present study, angle-independent indices were measured simultaneously with the absolute flow velocities by the inbuilt equipment software. The flow is at maximum velocity when the atrial pressure is minimum during the "S" and "D" peaks during ventricular systole and early ventricular diastole, respectively (Figure 1) and the flow is at minimum velocity when the atrial pressure is maximum during the "v" and "a" nadirs during end-systolic ventricular relaxation and atrial systole (Figure 1) (7). In daily practice, the PIV is clinically the most widely utilized (7). However, PIV is thought to give

Table 5. Predicted reference ranges for ductus venosus Doppler indices from 21 to 30 weeks of gestation

Week	Percentile	PIV	PVIV	a/S	S/a	Preload index	SIA index
21	5	0.36	0.34	0.34	1.48	0.32	0.64
	50	0.63	0.55	0.48	2.05	0.51	0.81
	95	0.85	0.74	0.67	2.89	0.65	0.97
22	5	0.32	0.29	0.33	1.42	0.29	0.61
	50	0.61	0.55	0.49	2	0.5	0.79
	95	0.87	0.75	0.7	2.99	0.67	1
23	5	0.37	0.34	0.33	1.48	0.32	0.65
	50	0.59	0.53	0.51	1.93	0.48	0.78
	95	0.88	0.79	0.67	2.96	0.66	1.03
24	5	0.38	0.35	0.42	1.51	0.34	0.65
	50	0.56	0.48	0.53	1.86	0.46	0.79
	95	0.73	0.65	0.65	2.36	0.58	0.89
25	5	0.33	0.3	0.3	1.42	0.29	0.64
	50	0.55	0.48	0.53	1.85	0.46	0.75
	95	0.9	0.78	0.7	3.23	0.69	1.07
26	5	0.28	0.26	0.36	1.35	0.26	0.63
	50	0.55	0.47	0.56	1.86	0.43	0.73
	95	0.82	0.7	0.73	3.51	0.64	1.01
27	5	0.37	0.34	0.32	1.48	0.32	0.65
	50	0.61	0.55	0.49	2	0.5	0.83
	95	0.93	0.76	0.67	3.07	0.67	1.05
28	5	0.24	0.23	0.42	1.29	0.23	0.59
	50	0.55	0.49	0.54	1.82	0.45	0.78
	95	0.73	0.64	0.77	2.34	0.57	0.94
29	5	0.32	0.28	0.43	1.4	0.28	0.63
	50	0.5	0.43	0.58	1.71	0.41	0.73
	95	0.73	0.72	0.71	2.33	0.56	0.92
30	5	0.26	0.24	0.33	1.32	0.24	0.61
	50	0.5	0.44	0.56	1.77	0.44	0.73
	95	0.92	0.77	0.75	3.01	0.66	1.07

PIV: Pulsatility index for veins, PVIV: Peak velocity index for veins, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, Preload index: (S-a)/S, SIA index: S/(a+v), Atrial preload index: (S+Ds)/(a+v), Atrial afterload index: (a+v)/(S+D)

Table 6. Predicted reference ranges for ductus venosus Doppler indices from 31 to 40 weeks of gestation

Week	Percentile	PIV	PVIV	a/S	S/a	Preload index	SIA index
31	5	0.29	0.26	0.42	1.36	0.26	0.61
	50	0.48	0.43	0.6	1.65	0.4	0.74
	95	0.73	0.65	0.73	2.36	0.57	0.89
32	5	0.32	0.29	0.34	1.4	0.29	0.63
	50	0.48	0.44	0.6	1.66	0.39	0.74
	95	0.84	0.72	0.71	2.92	0.65	0.92
33	5	0.15	0.14	0.34	1.17	0.14	0.56
	50	0.46	0.42	0.6	1.65	0.4	0.74
	95	0.89	0.8	0.85	2.92	0.65	1.02
34	5	0.21	0.2	0.35	1.24	0.19	0.58
	50	0.49	0.46	0.58	1.71	0.42	0.75
	95	0.86	0.73	0.8	2.79	0.64	1.04
35	5	0.2	0.19	0.36	1.24	0.2	0.57
	50	0.5	0.45	0.58	1.7	0.41	0.74
	95	0.83	0.78	0.8	2.76	0.63	1.02
36	5	0.15	0.14	0.38	1.16	0.14	0.56
	50	0.46	0.42	0.6	1.66	0.4	0.7
	95	0.79	0.73	0.85	2.63	0.61	0.94
37	5	0.11	0.1	0.39	1.11	0.1	0.54
	50	0.5	0.44	0.6	1.66	0.4	0.7
	95	0.84	0.74	0.89	2.52	0.6	0.95
38	5	0.14	0.13	0.36	1.15	0.13	0.56
	50	0.4	0.36	0.64	1.55	0.36	0.69
	95	0.83	0.76	0.86	2.71	0.62	1
39	5	0.09	0.08	0.34	1.1	0.08	0.53
	50	0.44	0.39	0.64	1.54	0.35	0.68
	95	0.94	0.76	0.9	2.87	0.65	1.06
40	5	0.18	0.17	0.44	1.19	0.16	0.55
	50	0.41	0.38	0.62	1.59	0.37	0.67
	95	0.72	0.7	0.83	2.23	0.55	0.89

PIV: Pulsatility index for veins, PVIV: Peak velocity index for veins, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, Preload index: (S-a)/S, SIA index: S/(a+v), Atrial preload index: (S+D)/(a+v), Atrial afterload index: (a+v)/(S+D)

an incomplete reflection of cardiac function, since relative changes in v- and D-wave velocities are not well represented (15) (Figure 1).

The afferent system of the fetal precordial venous system includes the intrahepatic UV, the LPV, the DV, the right portal vein (RPV) and the main portal vein. The PS is the L-shaped formation at the confluence of the LPV and RPV (20-22). The DV arises from the PS, just before turning at close to 90° to the right (Figure 11, 12) to create the pars transversa of the PS. The DV connects the LPV to the subdiaphragmatic vestibulum of the inferior vena cava, where the left and middle hepatic veins also connect (21,22). Maternal oxygenated blood in the LPV is shunted through the DV and directed towards the left atrium via the foramen ovale (18-20). The DV is an essential component of the classical "via Sinistra" pathway (22) and thus the myocardium and cerebral circulations receive more oxygenated and higher nutritional blood from the placenta.

Blood flow velocities of the DV in a cardiac cycle change according to the weeks of pregnancy because of some physiologic properties of the fetal circulation. For example, the human DV at 13-17 weeks of gestational age has novel structural features distinct from those of other blood vessels (23). Umbilical blood flow also decreases with gestational age and at 28 to 32 weeks, the shunting through the DV reaches the minimum (22,24). It has also been shown that the fraction of combined cardiac output directed to the placenta reduces after 34 weeks (24). When establishing reference ranges, the study population is of key importance, including when assessing reference values for blood flow velocities of the DV (25). The most difficult period to measure the patients was towards the end of pregnancy, especially between 38 and 40 weeks, since the proportion delivering by Caesarean section was much greater than those delivering by so-called "normal vaginal delivery", unfortunately.

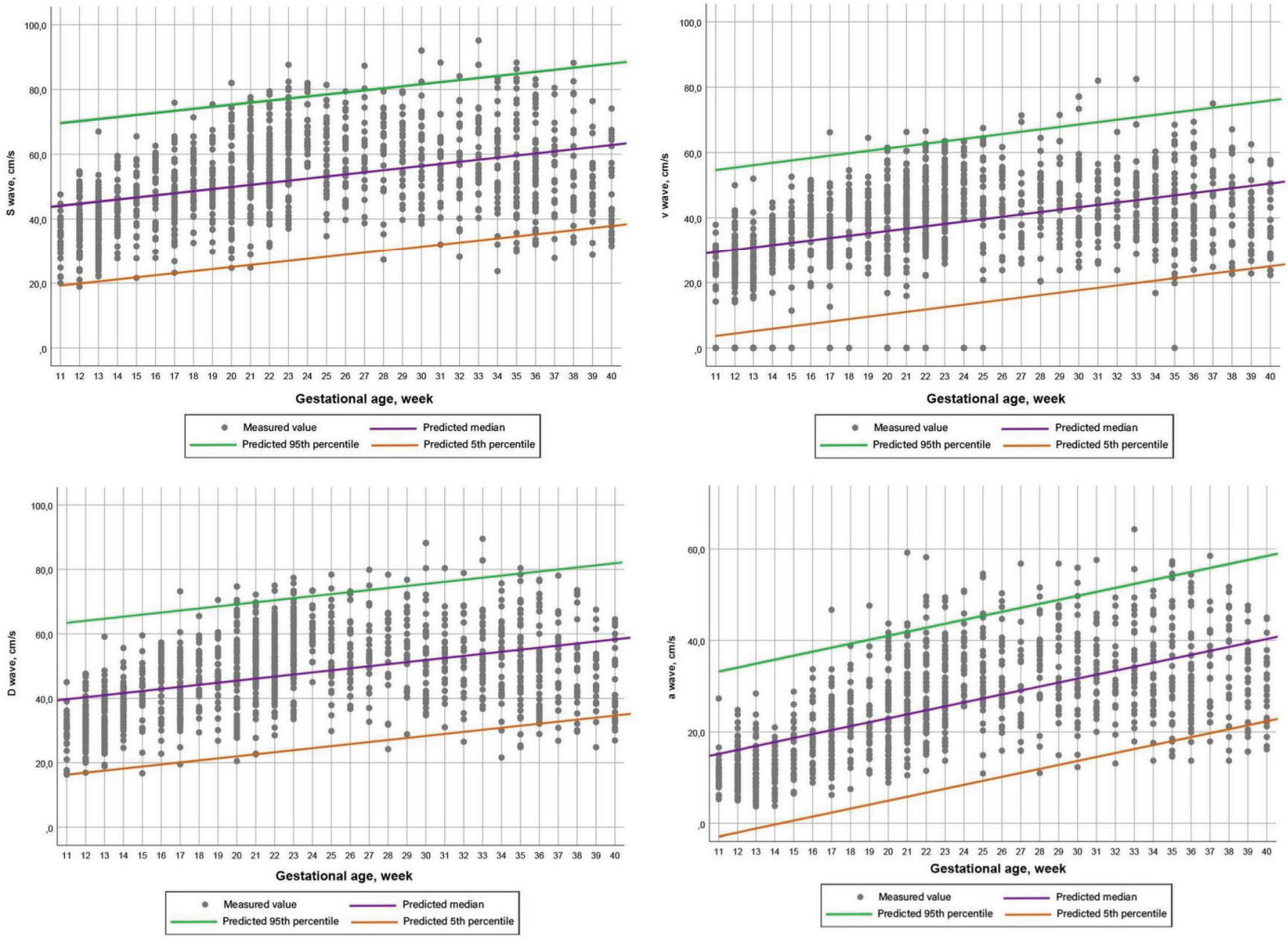

Figure 8. The scatter plots graphics for S, v, D, a wave (cm/s) against the gestational age

Study limitation

There are some limitations to the present study. The number of patients for each week of pregnancy was different, since the numbers of women with some indications are more likely to be scanned in a given set of gestational weeks than other weeks and timings. For example, patients in the first trimester are sent for the double screening test or in the second trimester undergo anomaly screening and are thus referred more frequently. However, the number of patients assessed in the present study was at least 25.

Turan et al. (3) studied the reference ranges of DV in 902 velocity wave ratios of pregnant women. The data was gathered retrospectively, obtained from two different fetal medicine centers, and scans were performed to a fixed protocol but by several sonographers and physicians. In addition, details of the ultrasonography equipment were also not. It is also well-known that ultrasonography is the most operator-dependent imaging modality. Therefore strengths of our study include: the largest patient population in the medical literature; being performed prospectively; using a single piece of ultrasonographic equipment with the same preset adjustments; being single center: and all scans were performed by a single specialist operator with experience in fetal imaging. The measurements were estimated either in the classic spectral Doppler pattern of the DV or, to the best of our knowledge, for the first time in the medical literature in the variants of the spectral Doppler waveforms, which were described recently (9). In contrast to earlier studies, the wideband color Doppler technique (ADF, Toshiba) was used to visualize the slow flow fetal vessels without blooming artefact, which was used to locate the DV and to place the sample gates. This technique allowed collection of faster spectral waveforms of the DV and more accurate and reproducible measurements.

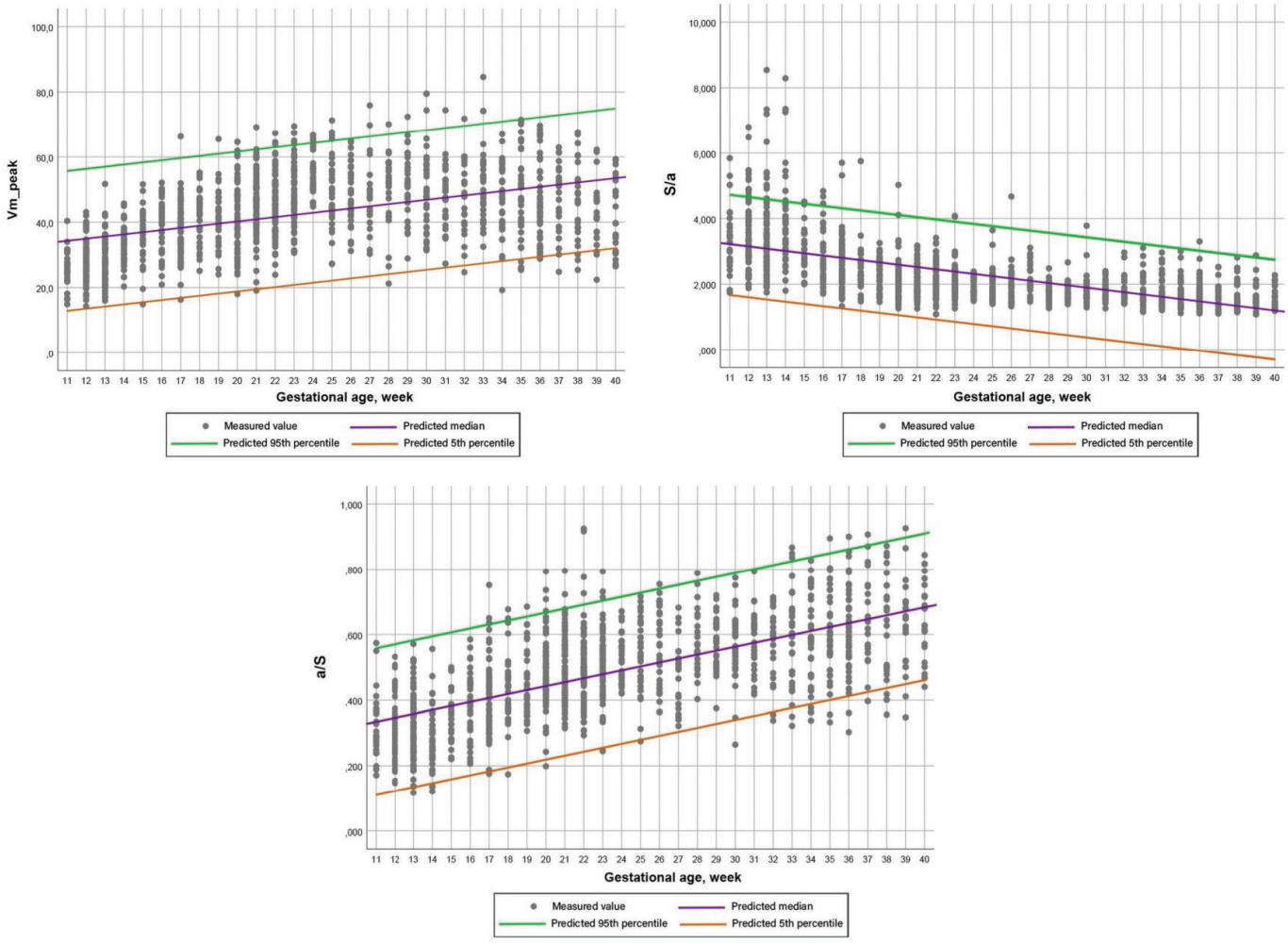

Figure 9. The scatter plots graphic for VmPeak wave (cm/s), S/a and a/S ratios against the gestational age

VmPeak: Time-averaged maximum velocity, S/a: The ratio of velocity in ventricular systole to velocity in atrial contraction, a/S: The ratio of velocity in atrial contraction to velocity in ventricular systole

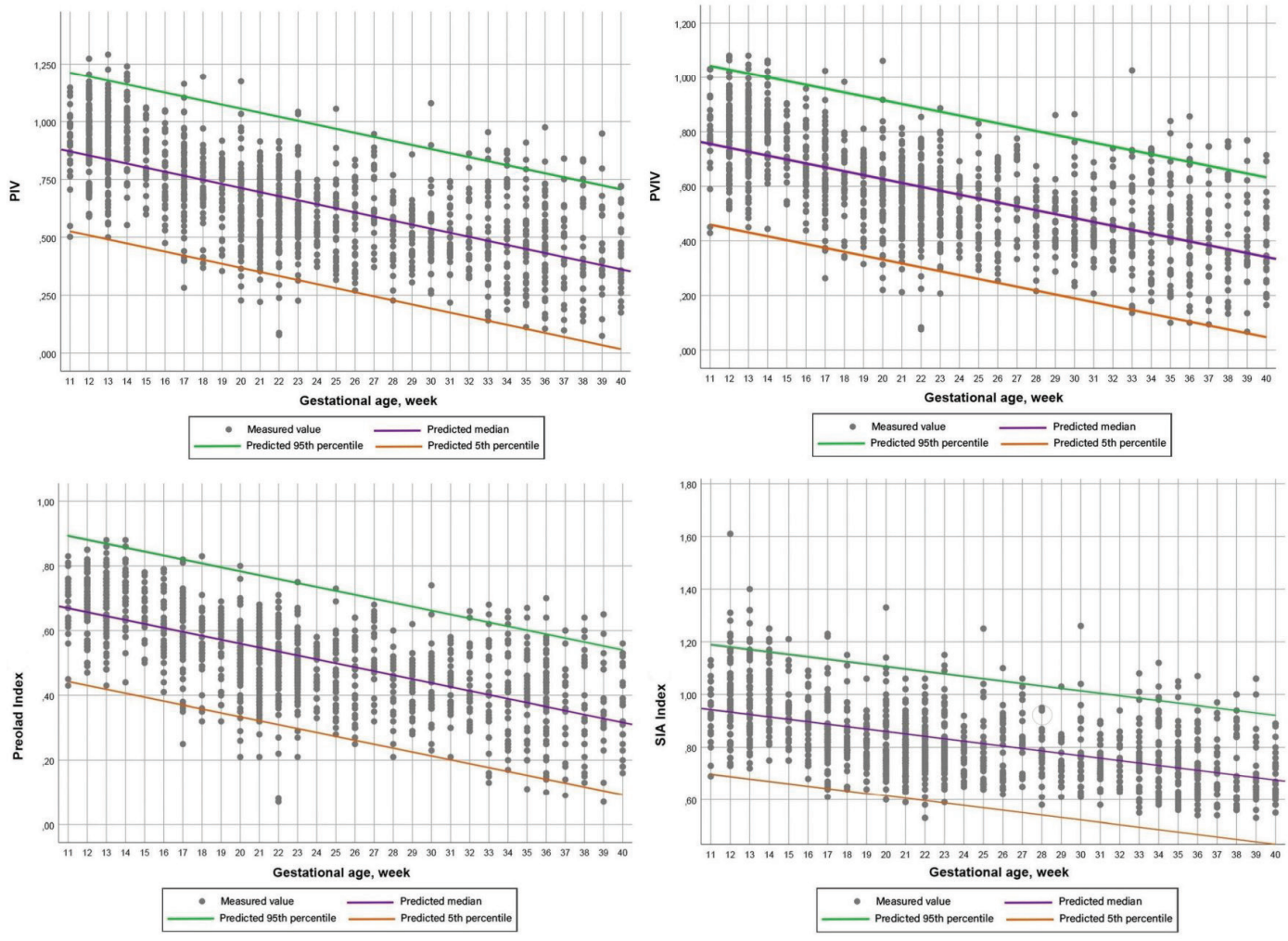

Figure 10. The scatter plots graphic for PIV, PVIV, preload and SIA indexes against the gestational age

PIV: Pulsatility index for vein, PVIV: Peak velocity index for vein, SIA: S/(a+v)

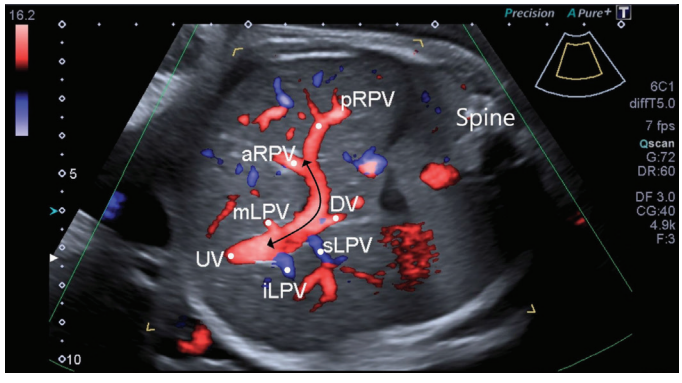

Figure 11. Afferent precordial venous system in an oblique axial color Doppler image

UV: Umbilical vein, DV: Ductus venosus, Curved black line: Portal sinus, mLPV: Medial branch of left portal vein, aRPV: Anterior branch of the right portal vein, pRPV: Posterior branch of the right portal vein

Conclusion

The advance in ultrasound technology has allowed easier mapping of the DV and collection of more reproducible measurements and so there was a need for re-establishment of reference ranges for DV velocities. This study has reported normal reference ranges for absolute flow velocities of S, v, D, a and VmPeak and indices, which were derived from velocities using a wideband Doppler technique, in the largest patient population so far. The study was undertaken prospectively in a single tertiary care center by a single experienced operator and can be used for detection and follow up fetal flow dynamics.

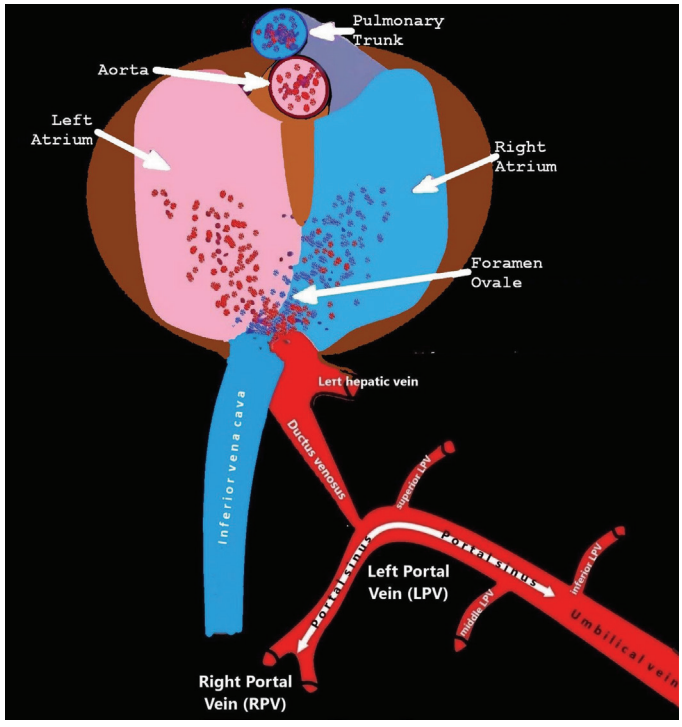

Figure 12. Flow distribution through the foramen ovale and the afferent precordial venous system in a painted illustration

Video 1. The spectral Doppler waveform of ductus venosus blood flow at 12 weeks gestation

https://www.doi.org/10.4274/jtgga.galenos.2021.2020.0232.video1

Video 2. The spectral Doppler waveform of ductus venosus blood flow at 34 weeks gestation in the oblique transverse plane

https://www.doi.org/10.4274/jtgga.galenos.2021.2020.0232.video2

Video 3. The spectral Doppler waveform of ductus venosus blood flow at 34 weeks gestation of the same patients in figure 6 in the sagittal plane

https://www.doi.org/10.4274/jtgga.galenos.2021.2020.0232.video3

Ethics Committee Approval: *The written informed consent from the patients and the ethical approval from the Ethics Committee of the University of Health Sciences Turkey, Antalya Training and Research Hospital were obtained (approval number: 16/13, date: 02.11.2017).*

Informed Consent: *The written informed consent from the patients were obtained.*

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Comparison of perioperative outcomes among robot-assisted, conventional laparoscopic, and abdominal/open myomectomies

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Abstract

Objective: To compare the perioperative results of myomectomy performed by robotic surgery (RM), laparoscopic surgery (LM), and open/abdominal surgery (OM).

Material and Methods: We included 227 patients who underwent either robotic (n=66), laparoscopic (n=88), or abdominal (n=73) myomectomy at our hospital between 2016 and 2020. Retrospective medical records, including fibroid characteristics, demographic findings, and surgical outcomes, were compared.

Results: The RM group had a significantly lower body mass index and significantly larger uterine size, myoma diameter, and myoma weight than the other groups. However, the OM group had the highest number of myoma. Moreover, the RM group had higher operative time and blood loss but significantly lower maximum visual analog scale values than the OM and LM groups. Hospitalization duration was significantly different among the groups. The rate of 1-day hospitalization was 56.2%, 64.8%, and 37.9% in the OM, LM, and RM groups, respectively. Furthermore, blood transfusion requirement was significantly higher in the OM group (12.3%) than in the LM and RM groups (0.0% and 4.5%, respectively).

Conclusion: Minimally invasive myomectomy may be preferable, particularly for women of reproductive age. In women with large uterine size and myoma, robot-assisted LM is recommended. (J Turk Ger Gynecol Assoc 2021; 22: 312-8)

Keywords: Robot-assisted surgery, laparoscopic surgery, open surgery, myomectomy

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Introduction

Myoma is a common problem among patients with benign gynecological disorders. Although the incidence of myoma is unclear in women of reproductive age, its frequency is approximately 5.4-70%, with manifestations including bleeding, gastrointestinal complaints, urinary complaints, pain, and infertility (1-3). Symptomatic myomas can be treated either medically or surgically. Surgical approaches include myomectomy, myolysis, endometrial ablation, hysterectomy, or uterine artery embolization (4). In cases where fertility sparing and surgical morbidity reduction are needed, myomectomy should be performed first before hysterectomy. Depending on the location, number, and size of myomas, and the skills of the surgeon, surgical options include hysteroscopic, robot-assisted laparoscopic, conventional laparoscopic, and abdominal/open myomectomy approaches. Minimally invasive surgery, including robot-assisted laparoscopy and conventional laparoscopy, reportedly has significant advantages, such as minimal bleeding, rapid recovery, short hospital stay, and less complication rates, compared with abdominal/open myomectomy (5-7). Despite some contraindications, minimal invasive surgery can be performed by experienced surgeons regardless of the size, number, or location of myomas (3,8). Studies have shown that robot-assisted surgery is superior to conventional laparoscopic surgery (LM) in terms of three-dimensional image provision, user-friendliness, and ergonomic position for the surgeon (9,10).

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In the present study, we aimed to compare the perioperative results of robot-assisted laparoscopic, conventional laparoscopic, and abdominal/open myomectomy cases in our hospital and compared this data with the data in literature.

Material and Methods

We included 227 patients who underwent either robotic (n=66), laparoscopic (n=88), or open/abdominal (OM) (n=73) myomectomy [robotic surgery (RM), LM, and OM groups, respectively] performed by the same surgeon (MG) at our hospital between 2016 and 2020. We retrospectively reviewed the medical records of these consecutive patients. We excluded patients who had undergone major surgeries other than myomectomy, had bleeding diathesis disorders, and had used gonadotropin-releasing hormone analogs preoperatively. Moreover, the size, weight, and location of the myoma; uterine size; symptoms; or previous abdominal surgery did not affect the eligibility criteria. However, we used robot-assisted LM for minimal surgery in fibroids of >20 weeks.

Myomectomy was indicated for pelvic pain, abnormal bleeding, abdominal mass, gastrointestinal symptoms, or genitourinary symptoms. After the surgical methods that can be applied according to the patient's indication and technical possibilities were explained in detail, the surgical method was decided in line with the patient's choice. The choice of the surgical route (RM, LM, or OM) was left to the discretion of the surgeon (MG) and patient preference. All procedures performed in this study conformed to the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the Medical Ethics Committee of the Institutional Ethical Review Board of the Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine (approval number: ATADEK-2020/27).

The operative time was defined as the time elapsed from intubation to extubation for each patient. The blood loss amount was defined as the total quantity of suction and irrigation. Furthermore, hospitalization duration was defined as the number of days from the day of surgery to the day of patient discharge.

In all LM and RM surgeries, patients were administered general anesthesia and were positioned in a lithotomy position with a steep (30°) Trendelenburg angle. Their arms were tucked with their palms facing toward lateral thighs with appropriate padding and legs were placed in booted stirrups. Pneumoperitoneum was then established up to 14 mmHg, with carbon dioxide insufflation throughout surgery.

In LM, a 10 mm 0° scope and three ancillary 5 mm ports (10 mm, umbilical; 5 mm, suprapubic; 5 mm, left; and 5 mm, right lower quadrant) were inserted. We used a 10 mm laparoscope and non-articulating instruments.

RM was performed using the da Vinci Xi Surgical System (Intuitive Surgical, Sunnyvale, CA). Three robotic arms (8 mm; umbilical, right ancillary port, and left ancillary) and a 12 mm assistant port with a smoke evacuator (Airseal®; SurgiQuest, Inc.) were used in all RM cases. Before robot docking, the procedure was initiated as a standard laparoscopy. During RM surgery, monopolar scissors and bipolar fenestrated forceps were used for dissection and vessel sealing, respectively. At the end of surgery, we switched back to laparoscopy for morcellation.

Before incision, desmopressin (30 μg/mL diluted in 100 mL of normal saline solution) was injected using a laparoscopic needle into the planned uterine incision site to decrease bleeding. We used Ultrasonic (Thunderbeat Olympus Medical Systems Corporation of America, 3500 Corporate Parkway, Center Valley, PA 18034, USA) and integrated advanced bipolar instruments for dissection and vessel sealing. After the incision, the exposed fibroid was enucleated by traction using grasping forceps. After fibroid removal, running sutures (Covidien 2-0 V-Loc™) were placed starting from the deepest myometrial layer, followed by multilayer closure. Finally, myoma tissues were excised using a power morcellator.

In OM, patients were administered general anesthesia and transverse suprapubic incisions were made. The uterus was then exteriorized. Before uterine incision, desmopressin (30 μg/mL diluted in 100 mL of normal saline solution) was injected into the incision site. In particular, we incised the uterine area overlying the myoma. The myoma was then enucleated. The myometrial layers were subsequently re-approximated in multiple layers using 1% absorbable polyglactin sutures in a running manner.

In all surgeries, the fascia and skin were closed layer by layer. Meanwhile, postoperative pain was assessed using the visual analog scale (VAS). VAS included distance (mm) measurement on the 10 cm line between the "no pain" anchor and the patient's mark and verbal descriptions (0 point, "no pain"; 10 points, "worst pain possible").

Statistical analysis

Continuous variables are expressed as means ± standard deviation and medians (minimum-maximum), whereas categorical variables are expressed as numbers or percentages where appropriate. In the intergroup analysis of continuous variables, data normality was analyzed using the Kolmogorov-Smirnov goodness-of-fit test. The continuous variables with normal distribution were then evaluated using One-Way analysis of variance (post hoc: least significant difference), whereas variables without normal distribution were compared

using the Kruskal-Wallis test (post hoc: Mann-Whitney U test) among the groups. Categorical data were compared using the chi-square test. All statistical data were analyzed using SPSS version 24.0 (IBM Corporation, Armonk, NY, USA), and the statistical significance level was set at $p<0.05$.

Results

A total of 227 patients were analyzed. Their mean age, parity, and previous cesarean section ratios were not significantly different among the groups ($p>0.05$). Compared with the other groups, the RM group had a significantly lower mean body mass index ($p=0.003$) and the highest mean week of gestation equivalent to the uterine size ($p=0.005$). However, the rate of history of previous surgery was significantly higher in the OM and LM groups (34.2% and 26.1%, respectively) than in the RM group (7.6%) ($p=0.001$). Bleeding (47.9%) and infertility (24.7%) were more common in the OM group, bleeding (35.2%) and pain (34.1%) were more common in the LM group, and bleeding (51.5%) and pelvic mass (22.7%) were more common in the RM group ($p<0.001$) (Table 1).

The myoma diameter (cm) and weight (g) were significantly higher in the RM group than in the other groups ($p<0.001$ and $p=0.001$, respectively), whereas the number of myoma was highest in the OM group ($p=0.002$). However, surgical location and pathology results were not significantly different among the groups ($p>0.05$) (Figure 1, 2, Table 2).

The RM group had higher operative time (minute) and more blood loss (mL) ($p>0.001$ for operative time, $p=0.098$ for blood loss) but had significantly lower maximum VAS scores ($p<0.001$) than the OM and LM groups. Furthermore, hospitalization duration was not significantly different among the groups ($p=0.013$). The rate of 1-day hospitalization was 56.2%, 64.8%, and 37.9% in the OM, LM, and RM groups, respectively. Blood transfusion requirement was significantly higher in the OM group (12.3%) than in the LM (0.0%) and RM groups (4.5%) ($p=0.002$). Abdominal drain requirement and complication rates were not significantly different among the groups ($p>0.05$). Median docking time and console time (15 and 140 minute, respectively) in RM were also noted (Figure 3, 4, Table 3).

In the LM group, one patient had postoperative fever and another patient had perineal edema. In the RM group, one patient had postoperative vomiting and another patient had ileus due to incisional hernia, requiring readmission. In the OM group, one patient had bladder injury. Meanwhile, conversion to laparotomy was not observed in the RM and LM groups.

Table 1. Comparison of demographic data and clinical findings

	OM (n=73)	LM (n=88)	RM (n=66)	Total (n=227)	p
Age (years)	38.88±5.28	38.01±5.42	38.61±5.76	38.46±5.47	0.590*
BMI (kg/m^2)	24.73±4.54	23.39±3.85	**22.55±2.52**[a]	23.57±3.85	**0.003***
Gestation equavalent to the uterine size	15.64±3.48	15.15±3.32	**16.78±2.50**[b]	15.78±3.22	**0.005****
Parity (n, %)					
Nulliparity	55 (75.3)	53 (60.2)	41 (62.1)	149 (65.6)	0.103***
Multiparity	18 (24.7)	35 (39.8)	25 (37.9)	78 (34.4)	
Previous cesarean section (n, %)					
Yes	62 (84.9)	72 (70.5)	46 (69.7)	170 (74.9)	0.056***
No	11 (15.1)	26 (29.5)	20 (30.3)	57 (25.1)	
Previous abdominal surgery history (n, %)					
No	48 (65.8)	65 (73.9)	61 (92.4)	174 (76.7)	**0.001*****
Yes	**25 (34.2)**	**23 (26.1)**	5 (7.6)	53 (23.3)	
Symptoms					
Pelvic pain	14 (19.2)	**30 (34.1)**	11 (16.7)	55 (24.2)	**<0.001*****
Abnormal bleeding	**35 (47.9)**	**31 (35.2)**	**34 (51.5)**	100 (44.1)	
Infertility	**18 (24.7)**	0 (0.0)	2 (3.0)	20 (8.8)	
Gastrointestinal symptoms	4 (5.5)	5 (5.7)	1 (1.5)	10 (4.4)	
Pelvic mass	1 (1.4)	17 (19.3)	**15 (22.7)**	33 (14.5)	
Genitourinary symptoms	1 (1.4)	5 (5.7)	3 (4.5)	9 (4.0)	
Total	73 (100.0)	88 (100.0)	66 (100.0)	227 (100.0)	

*One-Way analysis of variance (post hoc: [a]least significant difference), **Kruskal-Wallis test (post hoc: [b]Mann-Whitney U test), ***chi-square test, OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery, BMI: Body mass index

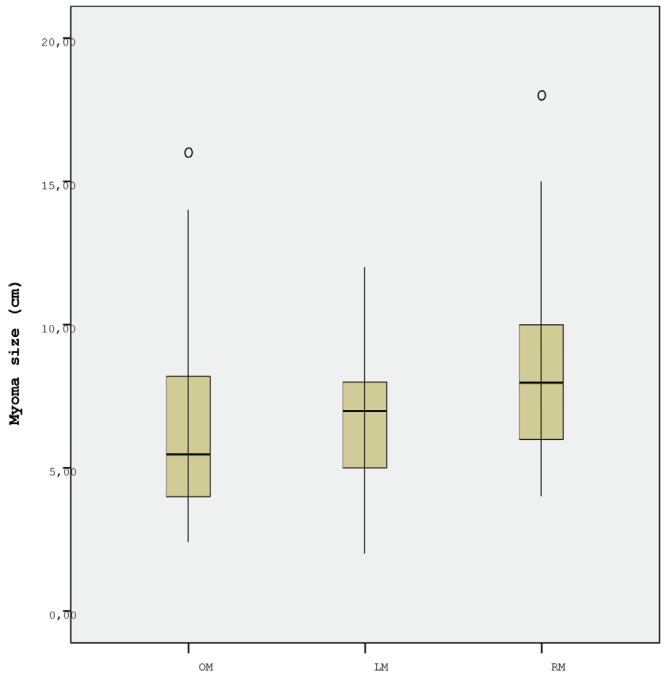

Figure 1. Box plot comparing size of the excised myomas as per surgical approach

OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery

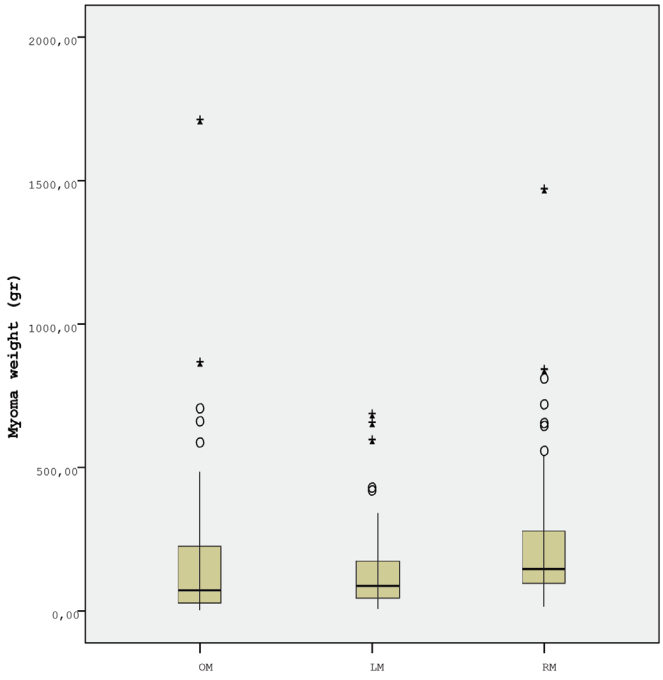

Figure 2. Box plot comparing weight of the excised myoma as per surgical approach

OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery

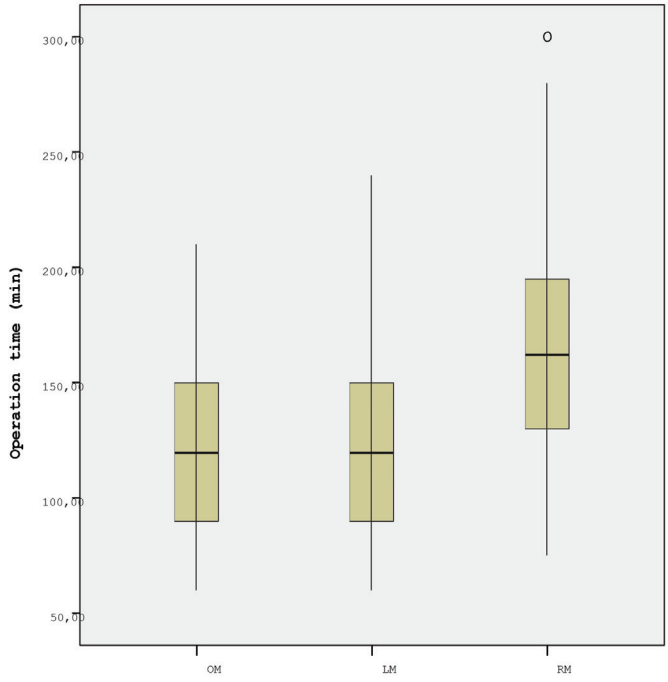

Figure 3. Box plot comparing operative time as per surgical approach

OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery

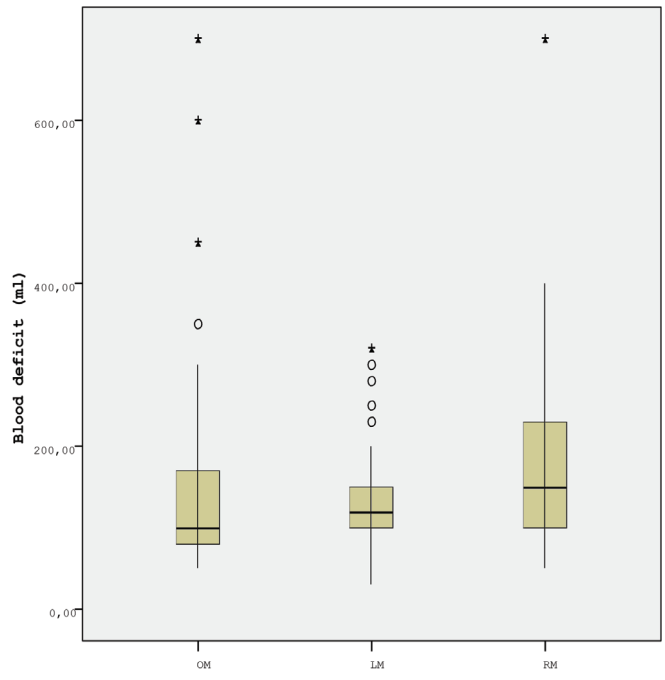

Figure 4. Box plot comparing blood loss as per surgical approach

OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery

Table 2. Myoma characteristics

	OM (n=73)	LM (n=88)	RM (n=66)	Toplam (n=227)	p
Size of largest myoma (cm) [median (min.-max.)]	5.5 (2.4-16)	7 (2-12)	**8 (4-18)[b]**	7 (2-18)	**<0.001***
Number of myomas [median (min.-max.)]	**4 (1-44)[b]**	3 (1-11)	3 (1-11)	3 (1-44)	**0.002***
Weight (g) [Median (min.-max.)]	75 (3-1,710)	90 (5-685)	**150 (15-1.469)[b]**	105 (3-1,710)	**0.001***
Location (n, %)					
Anterior	5 (6.8)	1 (1.1)	3 (4.5)	9 (4.0)	0.356**
Posterior	2 (2.7)	6 (6.8)	1 (1.5)	9 (4.0)	
Multiple	64 (87.7)	74 (84.1)	59 (89.4)	197 (86.8)	
Pedinculated	1 (1.4)	3 (3.4)	1 (1.5)	5 (2.2)	
Fundus	1 (1.4)	4 (4.5)	2 (3.0)	7 (3.1)	
Pathology (n, %)					
Leiomyoma	70 (95.9)	87 (98.9)	63 (95.5)	220 (96.9)	0.397**
Adenomyosis	3 (4.1)	1 (1.1)	3 (4.5)	7 (3.1)	
Total	73 (100.0)	88 (100.0)	66 (100.0)	227 (100.0)	

*Kruskal-Wallis test (post hoc: [b]Mann-Whitney U test), **: chi-square test, OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery, min.: Minimum, max.: Maximum

Table 3. Surgical factors and outcomes

	OM (n=73)	LM (n=88)	RM (n=66)	Total (n=227)	p
Operative time (minute) [median (min.-max.)]	120 (60-210)	120 (60-240)	**162.5 (75-300)[b]**	125 (60-300)	**<0.001***
Blood loss (mL) [median (min.-max.)]	100 (50-700)	120 (30-320)	150 (50-700)	120 (30-700)	0.098*
Maximum VAS [median (min.-max.)]	5 (2-9)	5.5 (2-9)	**3 (2-9)[b]**	5 (2-9)	**<0.001***
Docking time (minute) [median (min.-max.)]	-	-	15 (10-45)	-	-
Console time (minute) [median (min.-max.)]	-	-	140 (60-275)	-	-
Hospital stay (day) (n, %)					
1 day	**41 (56.2)**	**57 (64.8)**	25 (37.9)	123 (54.2)	**0.013****
2 days	25 (34.2)	27 (30.7)	**36 (54.5)**	88 (38.8)	
≥3	7 (9.6)	4 (4.5)	5 (7.6)	16 (7.0)	
Need for abdominal drain (n, %)					
No	69 (94.5)	88 (100.0)	64 (97.0)	221 (97.4)	0.095**
Yes	4 (5.5)	0 (0.0)	2 (3.0)	6 (2.6)	
Need for blood transfusion (n, %)					
No	64 (87.7)	88 (100.0)	63 (94.5)	225 (94.7)	**0.002****
Yes	**9 (12.3)**	0 (0.0)	3 (4.5)	12 (5.3)	
Complication (n, %)					
No	72 (98.6)	86 (97.7)	64 (97.0)	222 (97.8)	0.800**
Yes	1 (1.4)	2 (2.3)	2 (3.0)	5 (2.2)	
Total	73 (100.0)	88 (100.0)	66 (100.0)	227 (100.0)	-

*: Kruskal-Wallis test (post hoc: [b]Mann-Whitney U test), **: chi-square test, OM: Open/abdominal surgery, LM: Laparoscopic surgery, RM: Robotic surgery, min.: Minimum, max.: Maximum

Discussion

Considering that myomas are common in women of reproductive age, myomectomy is the gold standard in those who desire fertility. In this study, we evaluated the superiority of the different myomectomy methods by comparing the perioperative results of LM, RM, and OM cases in our hospital. Minimally invasive surgery, either LM or RM, has several advantages, including shortened hospitalization duration and reduced postoperative pain (8). Postoperative adhesion

rate also appears to be lower in LM, which is beneficial for reproductive age patients (11). Although uterine rupture during labor or pregnancy is one of the main concerns in this group of women, it appears to be an infrequent complication when LM is performed by skilled surgeons (4,12). One of the factors that facilitate uterine rupture is poor closure of the uterine incision (12). Hence, we perform multilayer closure at our hospital.

In a meta-analysis with 2,027 participants conducted in 2015 by Iavazzo et al. (13), the size of myomas was significantly larger in the OM and RM groups than in the LM group. Likewise, our study demonstrated that the RM group had a significantly larger myoma size and higher weight than the LM and OM groups.

Although significantly numerous myomas were excised in both the RM and OM groups in the study by Barakat et al. (14), significantly more myomas were excised in the OM group than in the RM and LM groups in our study. In minimally invasive surgery (RM or LM), extremely small intramural myomas may not be noticed when touched unlike in OM. This can explain the significantly higher number of myomas excised in the OM group.

In our study, the operative time was significantly higher in the RM group than in the OM and LM groups, although no difference was observed between the OM and LM groups. This finding is compatible with the findings of previous studies (13,15). In the study by Nezhat et al. (15), the operative time and cost of RM were significantly high, similar to our study. In another study, the blood loss amount and hospitalization duration were higher in the RM group (12). Processes such as setting up the RM device and switching to laparoscopy for morcellation after myoma excision may explain the longer operative time of RM than that of LM. Moreover, a larger myoma size and higher weight might explain the higher operative time in the RM group.

In the study by Barakat et al. (14), blood loss and blood transfusion requirement were less observed in the RM group, with 4.7% as the total blood transfusion rate in the entire cohort of their study, compared with 5.3% in our study. The RM group in our study had higher blood loss but had significantly less requirement for blood transfusion than the other groups.

In the study by Griffin et al. (7), pain scores were not different between the OM and RM groups. In our study, however, the postoperative pain score was significantly lower in the RM group than in the other groups. Thus, RM is more advantageous in terms of postoperative pain. Although postoperative fever can be observed after myomectomy (16), it was observed only in one patient among all groups in our study.

To minimize the risk of uterine rupture in pregnancy after myomectomy in women of reproductive age, the incision must be closed meticulously in a multilayer manner. Although the dexterity provided by the instruments in RM enables performing this closure more easily, its high cost, larger incisions, and longer operative time appear to be disadvantages that need to be overcome according to experience. In addition, conventional LM can be performed safely by skilled surgeons, thereby overcoming the disadvantages of RM.

Study Limitation

Some of the limitations of our study are its retrospective design and the failure to compare long-term outcomes, such as pregnancy rates, uterine rupture, and uterine adhesions. Moreover, the number and size of myomas excised were not similar in either of the groups. RM is preferred for myomas with large size and weight owing to the higher cost of the surgery at our hospital. Prospective, randomized trials on similar myoma size and number, as those in our study, with long-term outcomes are needed.

Conclusion

When performed by experienced surgeons, minimally invasive myomectomy (LM or RM) may be a good choice, particularly for women of reproductive age because of its several advantages, such as short hospitalization duration, less blood transfusion and drain requirement, and less postoperative pain. Although RM might not be preferred because of its long operative time, increased blood loss, and cost, it is preferable for patients with large myomas because it includes three-dimensional imaging, facilitates more precise surgery, and has significantly less postoperative pain.

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Perioperative considerations in the treatment of endometriosis

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Abstract

Endometriosis is one of the most common benign diseases in women of reproductive age. Nearly all gynecological offices and clinics will contain patients with endometriosis; the frequency and severity of the disease will vary from one setting to another. Adjoining specialties, such as internal medicine, general medicine, surgery, urology, orthopedics, neurology and psychosomatic medicine, will be challenged directly or indirectly by various forms of endometriosis and its sequelae. The disease is characterized by pelvic pain, dysmenorrhea, dyspareunia and sterility. Even now, several years may elapse between the onset of the disease and its diagnosis. The diagnosis of endometriosis is complicated by the diversity of the symptoms. A precise documentation of the patient's medical history and thorough diagnostic procedures are essential to establish a robust diagnosis. This article will discuss the perioperative considerations, diagnosis and treatment of endometriosis. (J Turk Ger Gynecol Assoc 2021; 22: 319-25)

Keywords: Deep infiltrating endometriosis, preoperative diagnosis, transvaginal ultrasound, adenomyosis, infertility

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Introduction

Endometriosis is one of the most common benign diseases in women of reproductive age. Non-specific symptoms, such as pain in the lower abdomen, dysmenorrhea, dyspareunia, bleeding disorders, cyclic micturition or defecation disorders, the unfulfilled desire to have children, or chronic fatigue are among the diverse pathomorphological symptoms and their location, as well as the manifold reactions of patients to the disease. Therapy options are equally diverse. These range from analgesia, watchful waiting and endocrine treatment to surgical strategies and combined procedures. The treatment should be aligned to the patient's condition (1-4). Quality of life is, in some cases, markedly impaired by endometriosis. The financial burden on health care systems and absenteeism from work are of significant socioeconomic significance. Patients with endometriosis have a diminished capacity for work: their average absence from work amounts to 7.41 hours per week (5).

Primarily due to lack of awareness of the disease, both on the part of patients and clinicians, an average period of 10.4 years elapses between the onset of non-specific symptoms and the establishment of the diagnosis. During this time, patients experience at least one erroneous diagnosis (6). As the symptoms may be quite general, false diagnoses, such as an irritable colon or pelvic inflammatory disease, are common (7). Similar numbers have been reported internationally with a mean diagnostic latency for endometriosis of 8 years in the United Kingdom and 11.7 years in the USA (8-11). In terms of pathogenesis, the disease is attributed to various factors, including retrograde menstruation, coelomic metaplasia, metastasis, altered cellular immunity, and a multifactorial mode of inheritance with interactions between the environment and specific genes (12-16).

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Symptoms

Characteristic symptoms of endometriosis include dysmenorrhea, dyspareunia, chronic pain in the lower abdomen, cyclic defecation or micturition disorders, sterility, or bleeding disorders. However, the pattern of symptoms may be quite ambiguous. Potential differential diagnoses (Table 1) must be noted and, if necessary, clarified by interdisciplinary consultation (17,18).

Pain intensity in endometriosis can be quantified by the use of a visual analog scale (VAS). The severity of pain using a VAS may be rated from zero (no pain) to ten (maximum pain). In addition to the intensity of pain, the patient should be asked about the duration of impairment in daily life, expressed in numbers of days per month. However, the clinician evaluating the symptoms should keep in mind the fact that the extent of disease or the size of the lesion is not always correlated with the intensity of pain (19).

Diagnosis

The patient's detailed medical history is complemented by a careful gynecological investigation. International and national guidelines demand a structured diagnostic approach (17,18).

Apart from inspection and palpation of the abdomen, the vagina should be investigated with a bivalve speculum in an appropriate setting. The bivalve speculum permits the detection of deep infiltrating lesions in the posterior vaginal vault. A bimanual palpation should also be performed. After informing the patient in advance, the clinician should perform a digital rectal examination, especially in cases of suspected deep infiltrating endometriosis. If possible, the surgeon himself/herself should perform the preoperative clinical investigation, record the patient's medical history (Table 2), and inform the patient about the subsequent procedure (3,17,18).

The combination of these actions is essential to obtain maximum information and minimize the risk of unexpected findings or omission of significant lesions. This approach also permits the identification of trigger points or the point of maximum pain, which yield crucial clinical data. These investigations are aided by an ultrasound investigation of the pelvic organs. In cases of suspected deep infiltrating endometriosis, an ultrasound investigation of the kidneys should be performed to rule out hydronephrosis (17,18).

Transvaginal ultrasound is the preferred diagnostic imaging procedure for the detection of endometriosis. It is widely available, economical, minimally invasive, and very informative in regard to deep infiltrating endometriosis, adenomyosis, or ovarian endometriosis (20). The published literature reports a sensitivity and specificity of 85% and 100%, respectively, for transvaginal ultrasound (21,22). In cases of deep infiltrating endometriosis, a magnetic resonance imaging investigation may optionally be performed as an additional imaging procedure. However, both methods yield similar results (20). Potential deep infiltrating nodules in the rectovaginal or vesicovaginal aspect can even be detected by transvaginal ultrasound. In cases of pronounced adenomyosis, these nodules may spread into the transuterine aspect or, independent of such infiltration, may also spread into adjacent regions. This must be included in the preoperative spectrum of endometriosis because surgical treatment may be difficult in these cases; patients should be informed preoperatively of the fact that dysmenorrhea or bleeding disorders may persist even after surgery. Furthermore, patients who wish to have children should be informed of the fact that adenomyosis may hinder conception (2,3).

Table 1. Differential diagnoses of acute and chronic pain in the lower abdomen in women of reproductive age (3)

Gynecological causes	Non-gynecological causes
Endometriosis	Acute or chronic appendicitis
Regular pregnancy	Nephrolithiasis
Intrauterine abortion	Perforation of a hollow organ (e.g. stomach, bowel, gallbladder)
Ectopic pregnancy	Obstruction of a hollow organ
Ovarian torsion	Intra-abdominal inflammation (peritonitis, diverticulitis, terminal ileitis, cholecystitis, gallstones)
Ovarian cyst	Rupture of a parenchymal organ (liver, spleen, kidneys)
Ruptured follicle or corpus luteum-cyst	Intra-abdominal infarction (bowel, mesentery)
Myoma	Internal bleeding
Adnexitis	Cystitis/pyelonephritis
Benign and malignant tumors of the inner genital organs	Benign and malignant tumors of the gastrointestinal tract

Table 2. Documentation of medical history in the presence of endometriosis (3,17,18)

Checklist-Essential questions regarding endometriosis
Pain shortly before or during menstruation
Cyclic symptoms and/or symptoms independent of the menstrual cycle
Pain during micturition or defecation
Blood in urine or stool
Pain during sexual intercourse
Unfulfilled desire to have children
Impaired quality of life
No symptoms

In cases of suspected deep infiltrating endometriosis and possible involvement of the bowel, a rectal endoscopic ultrasound investigation permits exact inspection and evaluation of the intestinal wall and its histological layers. This has far-reaching consequences for the treatment strategy (such as shaving versus partial bowel resection) (2,3,23). The primary purpose of preoperatively determined parameters is to aid the surgeon in estimating the extent of surgery and working out an individual therapy regimen, in consultation with the patient. The therapeutic strategy should take the patient's symptoms, wishes, emotional stress levels, the presence of limited organ function, and the reproductive aspect into account. Especially in cases of suspected deep infiltrating endometriosis and depending on the nature of preoperative findings, the surgical team should include a general surgeon skilled in endoscopy and a urologist (if necessary) in addition to the gynecologist. This is best achieved by referring the patient to a certified endometriosis center with expertise and specialized skills in the treatment of the disease (17,18).

Endometriosis and uterine malformations

The coexistence of endometriosis and uterine malformations, as shown in Figure 1a, b, which are frequently diagnosed during the exploration of infertility, has been reported by many authors (16,24-27). The underlying pathological mechanism could be intensified retrograde menstruation (27-29). This fact should also be included in preoperative considerations, especially in women who still desire to have children. Congenital uterine anomalies are more common than was previously assumed. Their clinical presentation depends on the anomalies and the woman's reproductive age. Some patients may be asymptomatic, with normal fertility and obstetric outcomes, while others may have primary amenorrhea, endometriosis, menstrual irregularities and infertility (16). The use of three-dimensional transvaginal ultrasound is extremely useful in these cases because it permits reconstruction of the uterine cavity and assessment of the external contours of the fundus (Figure 2a, b). The septate/subseptate uterus is the most common uterine malformation and is therefore of greatest significance in women who desire to have children (Figure 3) (16,25). Nawroth et al. (24), and recently LaMonica et al. (25) described a high rate of endometriosis in women with a septate uterus. Freytag et al. (16) showed that uterine malformations and adenomyosis frequently occur together, and their coexistence appears to be correlated with severe endometriosis. Therefore, endometriosis should always be suspected in patients with uterine malformations. Any surgical investigation of sterility should be performed as a combined hysteroscopy and laparoscopy (16).

Transvaginal ultrasound

In 2016, the International Deep Endometriosis Analysis group published a consensus paper with recommendations for specific diagnostic procedures in cases of suspected deep infiltrating endometriosis (30). A transvaginal ultrasound investigation is recommended in four steps of the examination procedure (30):

The first step is an evaluation of the uterus and uterine appendages. In addition to the mobility of the uterus, the myometrium should be inspected for sonographic signs of adenomyosis. The criteria specified by the Morphological

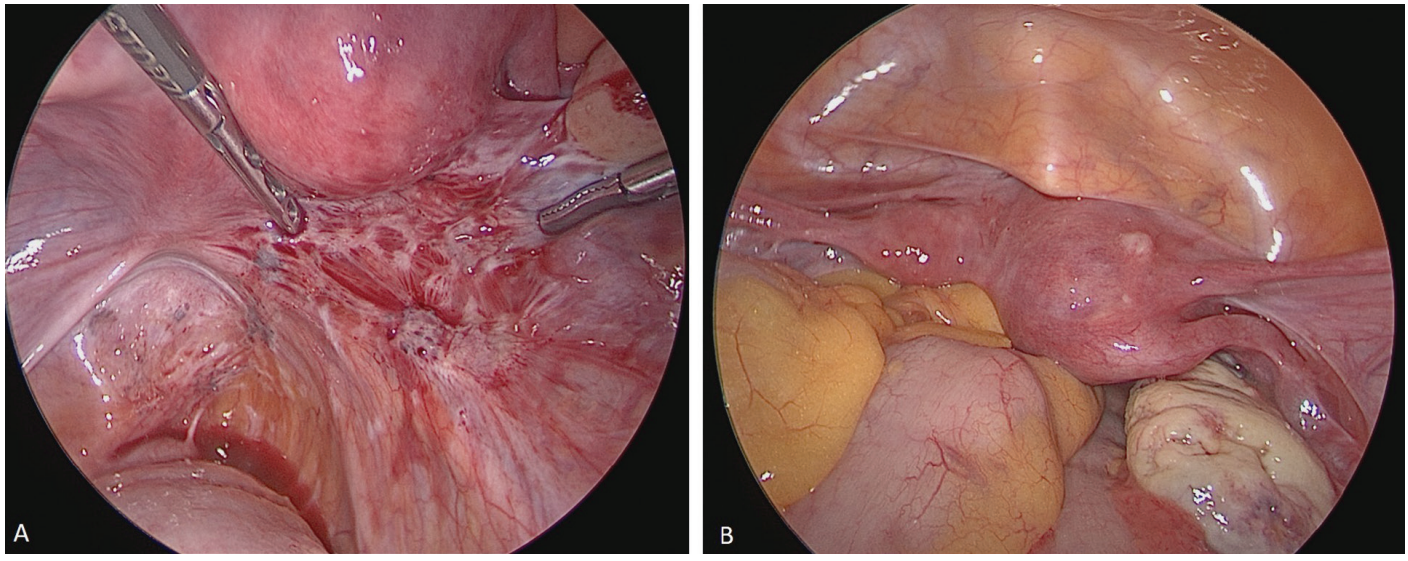

Figure 1. (A) Intraoperative findings in a 32-year-old patient with dysmenorrhea, dyspareunia and sterility. (B) In addition to deep infiltrating endometriosis, this patient has a uterine malformation by way of a unicornuate uterus with a non-communicating horn with functional endometrium. The fallopian tubes are seen here, and ligaments are inserted in the rudimentary horn

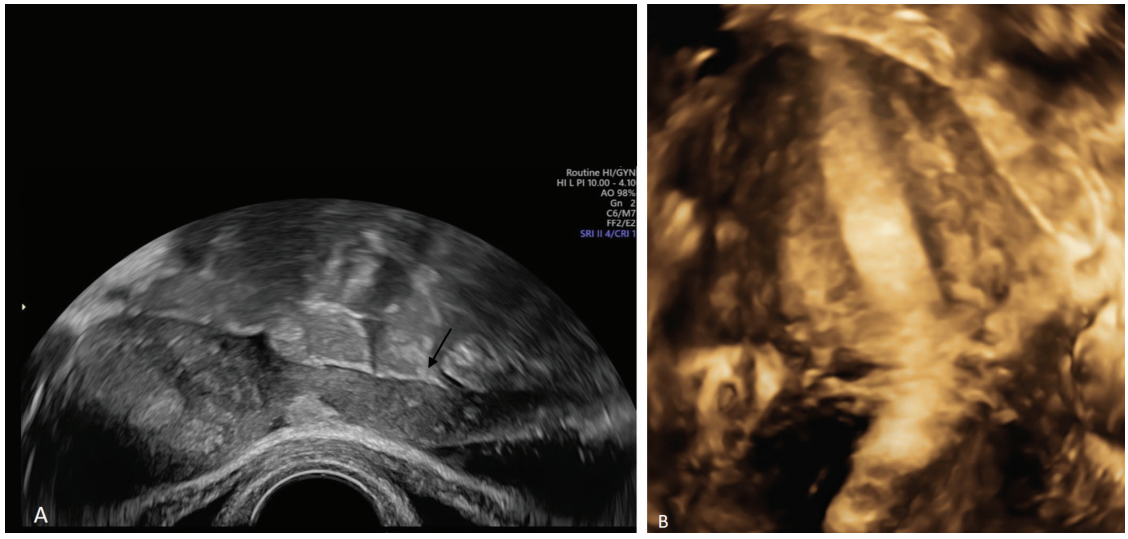

Figure 2. (A) Preoperative two-dimensional transvaginal ultrasound. The cross-section reveals a uterus deviated markedly to the left and a rudimentary horn with a small hyperechoic island of endometrial tissue. (B) Preoperative three-dimensional transvaginal ultrasound. The coronal plane reveals a markedly left-sided uterus and only one ostium of the fallopian tube and a narrow, elongated uterine cavity. The finding was confirmed on hysteroscopy. This patient has a non-communicating rudimentary horn

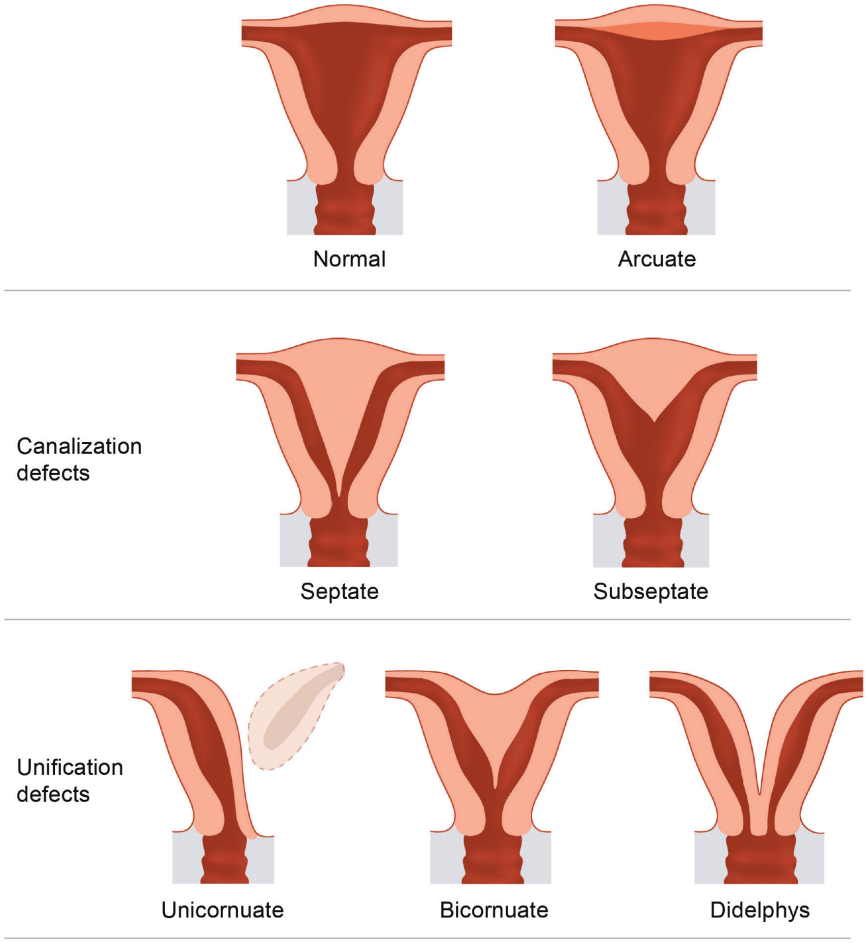

Figure 3. Schematic view of uterine malformations. The external contour of the uterus is colored orange. The uterine cavity is shown in red

Uterus Sonographic Assessment (MUSA) group should be used to describe these signs (31). The clinician looks for endometriomas in the uterine appendages, which should be described in accordance with the criteria given in the International Ovarian Tumor Analysis terminology (32). Endometriomas are usually unilocular cysts with ground glass echogenicity (30). A typical finding on color Doppler ultrasound is no, or minimal, vascularization of the cyst wall. These are usually associated with other endometriotic lesions particularly in the fallopian tubes, extensive adhesions, and deep infiltrating endometriosis. Mutually adherent retrouterine endometriomas and adherent ovaries are also referred to as kissing ovaries (Figure 4) (33). Kissing ovaries usually occur in conjunction with deep infiltrating endometriosis (33).

So-called soft markers are assessed in the second step of the investigation (30). Is the clinician able to locate painful regions? Are the ovaries movable (30).

The third step of the investigation includes a dynamic ultrasound examination for assessment of the so-called sliding sign. When the cervix, the posterior wall of the uterus and the fundus are movable, and the rectum and sigmoid colon can glide freely across the above-mentioned structures, the sliding sign is considered positive (30).

In the fourth step of the investigation, the clinician inspects the anterior and posterior compartments of the uterus in regard to deep infiltrating endometriosis. To locate the anterior compartment, which includes the bladder, the vesico-uterine pouch and the ureters, the ultrasound probe is placed in the anterior fornix. In addition to the mobility of the bladder (vesico-uterine adhesions), the investigator looks for deep infiltrating lesions. The bladder should be slightly filled with urine (30). Endometriotic lesions of the bladder are most frequently found in the posterior wall, followed by the base of the bladder (34). Visualization of the pelvic portion of the ureters is no substitute for an ultrasound investigation of the kidneys to exclude the presence of concomitant hydronephrosis (35). The posterior compartment is examined by placing the ultrasound probe in the posterior fornix. Here the clinician will be able to evaluate the sacrouterine ligaments, the vagina, the rectovaginal septum, the anterior rectum, the rectosigmoid junction, and the sigmoid colon. It should be noted that entities, such as the sacrouterine ligaments, may be visualized on transvaginal ultrasound only in the presence of pathological conditions (30).

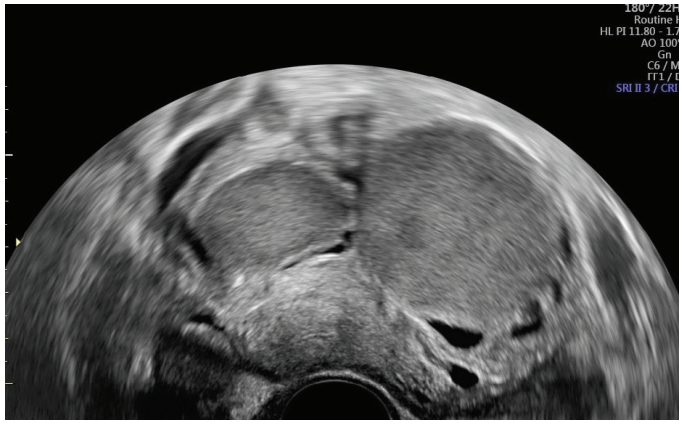

Figure 4. Ultrasound image of kissing ovaries with typical ground glass echogenicity

Adenomyosis: a special condition

Adenomyosis poses special problems for the managing physician in terms of diagnostic investigation and therapy. Patients are frequently young and still wish to have children. Therefore, hysterectomy is no option as a therapy of choice (36). Clinical symptoms of the disease include dysmenorrhea, menorrhagia, dyspareunia and pain in the lower abdomen. Furthermore, adenomyosis is a cofactor of female subfertility. The clinician must include this aspect in his/her preoperative considerations for the treatment of endometriosis, and counsel the patient accordingly (3).

A distinction is made between diffuse and focal adenomyosis. These are differentiated from adenomyomas. On histological investigation, adenomyomas are marked by additional compensatory hypertrophy of the surrounding myometrium (31). Differentiating this condition from myoma may be challenging, especially when both pathologies are present together. Color Doppler ultrasound may be useful in this setting. As mentioned earlier, the ultrasound investigation of adenomyosis should be performed in accordance with the MUSA criteria (31). Ultrasound findings (Figure 5, 6) that indicate the presence of adenomyosis include an asymmetrical thickening of the wall, so-called striae-like vascular patterns, fan-shaped shadowing, myometrial cysts, hyperechoic islands, echogenic buds and strips, and an irregular or interrupted junctional zone. The latter can be visualized well with the aid of three-dimensional transvaginal ultrasound in the coronal plane (31).

Conclusion

An exact documentation of the patient's medical history and careful diagnostic investigation with the aid of transvaginal ultrasound are prerequisites for planning effective treatment in patients with endometriosis. The diagnostic investigation must be based on profound knowledge of the typical symptoms of the disease, which include dysmenorrhea, dyspareunia, chronic pain in the lower abdomen, cyclic defecation or micturition disorders, sterility, or bleeding disorders. Patients should be referred to a certified endometriosis center for diagnostic investigation and treatment.

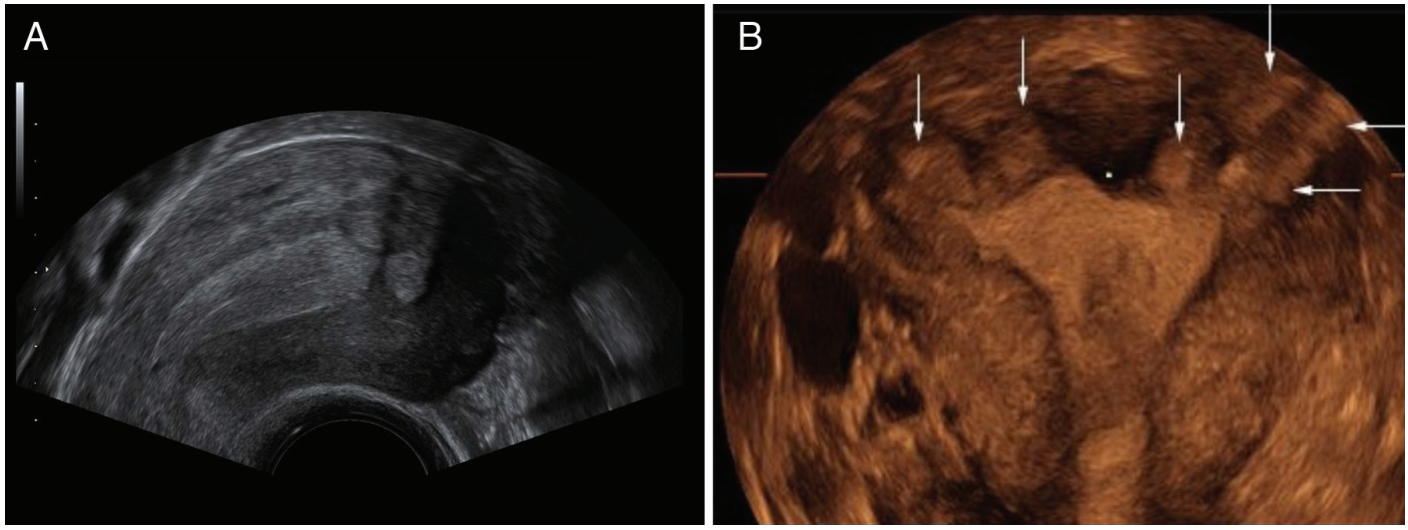

Figure 5. (A) This ultrasound image - a sagittal section through the uterus - shows adenomyotic lesions close to the endometrium. Characteristically, these lesions have the same echogenicity [extracted from (37)]. (B) In the coronal plane the three-dimensional transvaginal ultrasound investigation shows several adenomyotic lesions arising from the endometrium close to the fundus [extracted from (37)]

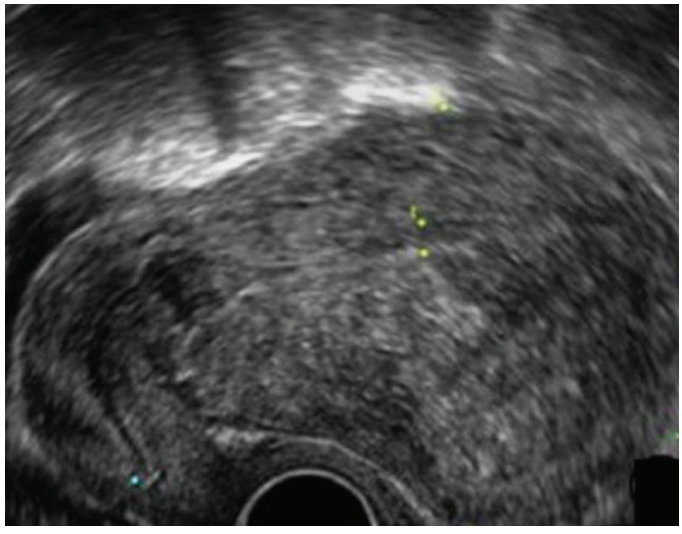

Figure 6. This sagittal section through the uterus shows the frequently encountered asymmetry of the uterine walls in the presence of adenomyosis

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An overview of polycystic ovary syndrome in aging women

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Abstract

The manifestations of polycystic ovary syndrome (PCOS), a ubiquitous reproductive disorder, may vary significantly depending on the severity of a number of endocrine and metabolic changes. Although no diagnostic criteria are presently available for PCOS for perimenopausal and menopausal women, the condition can still be suspected in case of a previous diagnosis of the condition, a chronic history of irregular menstrual cycles and hyperandrogenism, and/or polycystic ovarian morphology during the reproductive period. PCOS is associated with long-term health risks, including obesity, diabetes, hypertension, dyslipidemia, metabolic syndrome and cardiovascular risk factors during reproductive age, especially in patients possessing classic phenotypes. The aim of this review was to outline the available data about the impact of PCOS on long-term health risks after reproductive age in patients with PCOS. Previously, it was assumed that women with PCOS would be more prone to develop cardiometabolic diseases after reproductive age but current data suggest that in accordance with the healing in the phenotypic characteristics of PCOS, no deterioration appears to occur in cardiometabolic health in these patients. While there is substantial evidence for a greater prevalence of abnormal subclinical atherosclerotic markers among younger patients with PCOS, data for older women are insufficient. However, there is also support for an increased risk of endometrial cancer in PCOS patients. Extensive prospective cohort studies in which healthy controls as well as patients with defining PCOS phenotypes are observed and monitored from the early reproductive period into the late postmenopausal period should now be performed in order to clarify morbidities and mortality in aging women with PCOS. (J Turk Ger Gynecol Assoc 2021; 22: 326-33)

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Introduction

Polycystic ovary syndrome (PCOS) is the most frequently seen endocrine disorder among women of reproductive age, with a reported prevalence ranging between 5% and 20%, due to various diagnostic criteria employed (1-3). PCOS is a heterogeneous disorder defined by a combination of clinical or biochemical hyperandrogenism (HA), oligo-anovulation (OA) and polycystic ovarian morphology (PCOM) on ultrasound (4). Three sets of proposed diagnostic criteria are available for defining PCOS, produced by the National Institutes of Health (NIH), and the Rotterdam and AE-PCOS Society guidelines (5-7). In all three sets, other mimicking entities, such as thyroid disorders, hyperprolactinemia, hypercortisolemia and congenital adrenal hyperplasia, must be excluded before a diagnosis of PCOS is made. According to the Rotterdam criteria, at least two of the following are required for a diagnosis of PCOS-OA, HA, or the presence of PCOM (6). Various phenotypes have been identified based on these diagnostic characteristics. Phenotype A, in which patients satisfy all three PCOS diagnostic criteria, is the most common form. Patients with HA and OA but without PCOM are classified as Phenotype B, and those with HA and PCOM but not OA are classified as phenotype C. Phenotype D is a non-hyperandrogenic form including OA and PCOM (8).

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Women with PCOS may present with hirsutism, acne, irregular menses, and infertility during reproductive age. However, definition of PCOS in menopausal women is problematic. A previous history of OA, infertility, and HA have been employed in order to identify the phenotype for postmenopausal women in previous studies (9). PCOS is a life-long disorder entailing several long-term health risks (Figure 1). With increasing age, it was assumed that PCOS evolved from a reproductive disease to a more metabolic disorder including visceral obesity, dyslipidemia, diabetes mellitus (DM), hypertension (HT), metabolic syndrome (MS), cardiovascular diseases (CVD), and endometrial cancer (EC) (3). Limited evidence is available concerning the natural history of PCOS during the perimenopausal and menopausal period (10). This review discusses these changes and comorbidities after reproductive age in patients with PCOS in the light of the latest evidence.

Perimenopausal/menopausal transition in PCOS

Menopausal transition is a physiological process associated with aging and various associated hormonal and metabolic changes (11). Androgen levels remain stable or may even rise as menopause commences, while a marked decrease occurs in estrogen levels. Ovarian granulosa cells, the principal secretors of estradiol and inhibin, also decline. This reduced inhibition by estrogen and inhibin on gonadotropins results in increased secretion of these hormones. Antral follicule count and ovarian volume decline with age, eventually becoming incapable of responding to the effects of follicle-stimulating hormone. As ovarian aging progresses, estrogen levels may be quite variable, with chaotic patterns. In general, menopausal transition is characterized by a gradual decrease in menstrual bleeding. However, some women do experience heavy or prolonged bleeding, which has always been assumed to be due to anovulatory cycles and prolonged exposure to unopposed estrogen. The propensity for anovulatory cycles may lead to endometrial hyperplasia or carcinoma, and uterine polyps. After sometimes years of menstrual irregularity, women eventually experience permanent cessation of menses.

PCOS can be difficult to diagnose during the perimenopausal period, since aging leads to alterations in all three diagnostic criteria. The PCOS phenotype improves with age, as defined by an increase in regular menstrual cycles and decreased ovarian volume and follicle numbers (9). The Endocrine Society guideline suggests that a presumptive diagnosis of PCOS in older women might be based upon an appropriately evidenced long-term history of OA and HA during reproductive age (12). Elting et al. (13) demonstrated that women with PCOS frequently gain regular menstrual cycles due to loss of follicles during ovarian aging. In another study older women with PCOS who gained regular menstruation were compared with individuals whose cycles became irregular. The findings of this study showed that a lower follicle count in patients with PCOS predicted the emergence of regular menstrual cycles

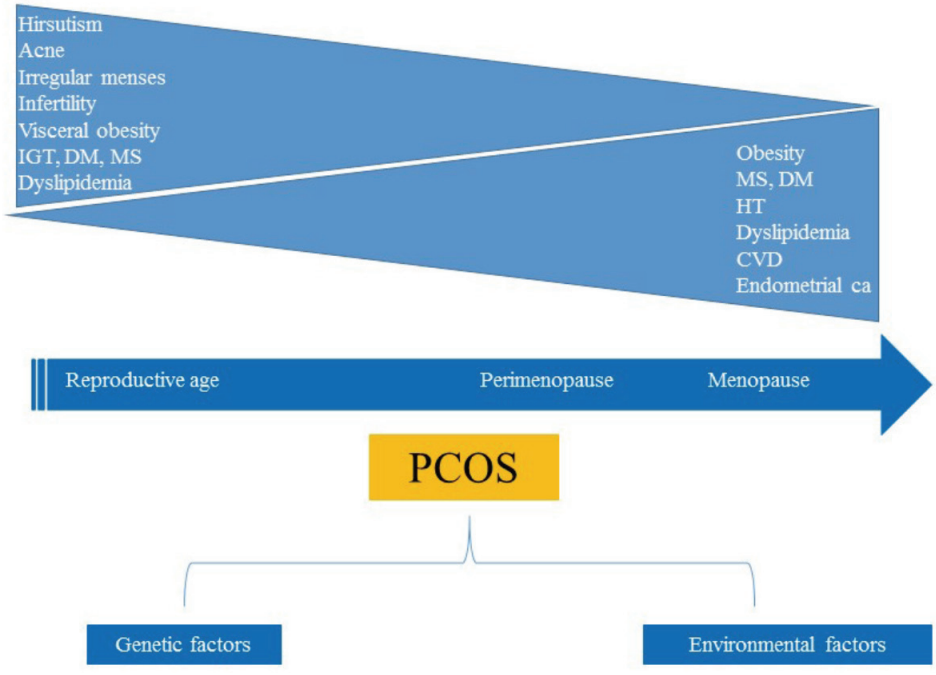

Figure 1. Clinical components of polycystic ovary syndrome throughout life

IGT: Impaired glucose tolerance, DM: Diabetes mellitus, MS: Metabolic syndrome, HT: Hypertension, CVD: Cardiovascular diseases, PCOS: Polycystic ovary syndrome

with age. This in turn confirmed that the regular menstrual cycles observed among older women with PCOS is principally due to a reduction in the follicle cohort size with ovarian aging (14). Alsamarai et al. (15) suggested that women with PCOS exhibited a less marked decrease in ovarian volume than healthy controls. A combination of age, and a decrease in log ovarian volume, follicle number, and testosterone (T) levels have been proposed for differentiating PCOS in women over 40 from healthy controls (15).

HA is of central significance in the pathophysiology of PCOS and is linked to anovulation, infertility and several metabolic diseases. Partial resolution of HA may be observed in the perimenopausal period among patients with PCOS due to ovarian and adrenal aging and reduced androgen production (10). In a study of 84 women with PCOS, Winters et al. (16) reported approximately 50% lower total T and non-dehydroepiandrosterone sulfate (DHEAS)-bound T-levels in the 45-47 age group compared to those in women in their 20s and 30s (16). Carmina et al. (17) followed-up 193 women, from a mean age of 22 to 43, and reported decreases of approximately 25% in T-levels and 30% in DHEAS levels. Ovarian size also decreased, by approximately 20%, in women with PCOS after long-term follow-up, and more ovulatory cycles were observed, indicating a milder disorder. Although the majority of women with mild HA eventually normalized, many others with the more severe phenotypes (A and B) remained hyperandrogenic (17). However, other authors have reported that overproduction of androgen levels in women with PCOS persisted even after menopausal transition (18). Markopoulos et al. (19) observed higher 17-hydroxyprogesterone, Δ4-androstenedione (Δ4A), DHEAS, total T, and free androgen index (FAI) levels and baseline lower sex hormone binding globulin levels in postmenopausal patients with PCOS compared to control subjects. These authors inferred from this that postmenopausal women with PCOS are exposed to higher androgen levels, both adrenal and ovarian, than individuals without PCOS (19). Pinola et al. (20) reported elevated serum androgen levels even in the postmenopausal period in women with PCOS. Calculated free T, Δ4A and FAI were identified as the most accurate predictors of PCOS at all ages (20). Puurunen et al. (21) reported that basal androgen, with the exception of DHEA, either remained unchanged or else was slightly higher in pre- and post-menopausal women with PCOS, and that area under the curve levels were also higher. In another study, increased ovarian androgen levels were reported, together with adverse metabolic changes including impaired glucose tolerance (IGT) and chronic inflammation among premenopausal women with PCOS, and that these findings continued after the perimenopausal period. These results were described as emphasizing life-long health risks associated with PCOS (22). Hirsutism is more prevalent in women with PCOS than in the normal healthy population, although data concerning acne and alopecia in these patients are lacking (23). Liang et al. (24) showed that androgens (TT and DHEAS levels), modified Ferriman-Gallwey (mFG) score and the prevalence of acne and hirsutism decreased with increasing age, but also reported that visceral obesity and various metabolic disorders were significant concerns in aging women with PCOS.

In terms of menstrual cycles, the average age at menopause in women with PCOS is unclear. Anti-mullerian hormone (AMH) level may be a beneficial predictor of age-related ovulatory function among patients with PCOS with anovulatory cycles (25). One study, which used AMH as a predictive indicator, reported women's reproductive lifetime with PCOS lasted a mean two years longer than in normo-ovulatory individuals (26).

Long-term complications of aging women with PCOS

Obesity

The incidence of obesity, including visceral obesity, rises after menopause. This in turn encourages a number of metabolic disorders, including MS, diabetes and atherosclerosis (11). Visceral obesity is a frequently seen characteristic of PCOS, and one that exacerbates the severity of both reproductive and metabolic problems. Although several studies and meta-analyses showed a higher prevalence of overweight and obesity in women with PCOS than in women without PCOS, the data in aging women with PCOS are insufficient (9,27). Meun et al. (28) recently observed higher body mass index (BMI) values and increased waist circumferences in women with PCOS aged >45 years than in healthy controls. Wild et al. (29) reported significantly higher BMI and waist-to-hip ratio (WHR) values in a PCOS group than in a control group in a 31-year follow-up study. The Rotterdam study reported higher BMI and WHR in patients with PCOS than in age-matched controls (30). However, a 21-year follow-up study of patients with PCOS in the 61-79 age group (n=25) determined no differences in terms of BMI or WHR between patients and age-matched controls (n=68) (31). Echiburú et al. (32) investigated women with PCOS and healthy controls in three separate periods of reproductive life; women aged 18-34 years, 35-40 years and between 41-55 years. BMI and WHR values were higher among patients with PCOS in the early and late reproductive periods, but no difference was observed between the patients and controls in the perimenopausal period. Only homeostasis model assessment-β levels were lower in the late reproductive and perimenopausal periods in the patients with PCOS compared to early reproductive age (32). Overall most studies, but not all,

support the idea that women with PCOS at older ages persist in being more overweight/obese than healthy controls.

Metabolic syndrome

There is increased prevalence of MS associated with central obesity, insulin resistance, and HT in women after menopause. However, limited data are available concerning the prevalence of MS in women with PCOS during the perimenopausal period (9,10). Pinola et al. (20) reported a two- to five-fold higher prevalence of MS in women with PCOS compared with healthy controls, depending on age and phenotype. The highest prevalence was determined in hyperandrogenic women with PCOS towards the end of reproductive age (20). The Study of Women's Health Across the Nation (SWAN) study indicated no association between a history of HA or menstrual irregularity and impairment of metabolic condition after menopause (33). Meun et al. (28) did not observe significant difference in the prevalence of MS between women with PCOS and controls at the age of 50. Overall, women with PCOS experience a higher risk of MS during the reproductive period. However, with menopausal transition, the risk of MS becomes similar to that of women without PCOS.

Impaired glucose tolerance and type 2 diabetes mellitus

Studies have reported a greater prevalence of IGT and type 2 DM, independent of BMI, in women with PCOS (34). The SWAN study demonstrated a higher prevalence of IGT among women with PCOS with a mean age of 45.8 years compared to control subjects (25% vs 9.2%, respectively, $p<0.001$) (33). Several long-term studies have identified PCOS as a risk factor for DM (35). Metabolic changes, such as insulin resistance, persist beyond menopause in women with PCOS, thus increasing their susceptibility to DM. Several studies have investigated the relationship between PCOS and DM in aging women (31,36,37). Wang et al. (38) reported a two-fold greater probability of DM incidence over a period of 18 years in women with PCOS in the CARDIA study. The probability of DM incidence was three times greater in normal-weight women with PCOS compared with weight matched healthy controls. The highest odds of diabetes [odds ratio (OR) 7.2, confidence interval (CI) 1.1-46.5] was detected among women with persistent PCOS fulfilling NIH criteria at baseline and during follow-up (38). A retrospective cohort study from the UK reported an age-dependent increase in the prevalence of DM, rising from 4.4% at ages 16-44, to 11.1% at 45-54 years, 15.7% at 55-64 years, and 45.4% at age >65. Although the OR of DM in PCOS was higher in all groups compared with age-matched women from the Health Survey for England, the lack of adjustment for BMI in that study was described as a significant limitation (39). A prospective, follow-up study from Northern Finland reported a greater probability of DM at 46 years among women with presumed PCOS than in healthy control women (12.4% vs 4.3%; $p<0.001$). Those authors reported that PCOS significantly enhanced the risk of DM in overweight/obese (BMI $\geq$25.0 kg/m^2) women with PCOS compared to weight matched healthy controls (OR: 2.45, 95% CI: 1.28-4.67), but not in normal-weight women (40). In a longitudinal study employing the Taiwan National Health Research Database, Lin et al. (41) observed that women with PCOS exhibited an increased risk of obstructive sleep apnea development in later life. They also found a greater prevalence of dyslipidemia (3.1% vs 2.4%, $p=0.049$) and DM (2.4% vs 1.4%, $p=0.001$) among individuals with PCOS compared to a control group (41). A higher prevalence of DM was also reported in the Rotterdam study among women with presumed PCOS compared to healthy control individuals (18.9% vs 7.0%, respectively, $p<0.01$). However, again no adjustment was made for BMI or WHR when assessing risk of DM (30). Wild et al. (29) observed a higher prevalence of DM among women with PCOS than among age-matched controls (6.9% vs 3.0%, respectively, mean age 56.7 years), although that significant difference was no longer apparent following adjustment for BMI. Schmidt et al. (31) observed no significant difference in the prevalence of DM at 61-79 years of age, which may have been associated with the low numbers of participants and the relatively small PCOS population enrolled. Merz et al. (42) also determined no significantly greater prevalence of DM among postmenopausal women exhibiting clinical characteristics of PCOS in their long-term follow-up study. Finally, a cross-sectional study using the Dallas Heart study data, also observed no significant differences in terms of proportions of women with DM (36).

Limited evidence suggests that women with PCOS have an increased risk of DM in the perimenopausal period, and a higher risk of IGT during the reproductive period. The current recommendation is that all women with PCOS should be screened for DM at the initial visit, irrespective of age and BMI (12,23). Further research is now needed to illuminate this association after adjustment for risk factors including BMI and family history in older PCOS patients.

Dyslipidemia

Dyslipidemia is the most frequently seen metabolic disturbance in patients with PCOS, at rates as high as 70% according to National Cholesterol Education Program guidelines (12). Hypertriglyceridemia, low high-density lipoprotein (HDL)-cholesterol and high low-density lipoprotein (LDL)-cholesterol levels have been reported in both obese and lean women with

PCOS in previous studies (12,43,44). This atherogenic lipid profile derives from insulin resistance and HA, together with various genetic and environmental factors such as diet and lack of physical exercise (23). The most powerful evidence of an association between menopause and adverse cardiovascular risk alterations is this data showing pro-atherogenic changes occurring in the lipid profile (45). However, a small number of studies have investigated the prevalence of dyslipidemia in aging patients with PCOS (9,10). Wild et al. (43) reported lower HDL-cholesterol level, higher levels of TG, LDL-cholesterol and non-HDL cholesterol, independent of BMI, in women with PCOS. The CARDIA study showed a two-fold greater risk of incident dyslipidemia among women with PCOS during 18 years of follow-up (38). Pinola et al. (20) compared women with PCOS (normoandrogenic-NA or hyperandrogenic-HA) to healthy controls. These were classified under three age groups: <30, 30-39, and >39 years. In the HA-PCOS group women over 39 years-old exhibited higher LDL and triglyceride levels compared with the controls and higher LDL levels compared with the NA-PCOS population after adjustment for BMI. However, several studies showed no significant difference regarding dyslipidemia in aging women with PCOS (28,32,37,42). Meun et al. (30) reported lower HDL and higher triglyceride levels in women aged >55 years with PCOS. Schmidt et al. (31) observed that the levels of TG and LDL had increased, while HDL levels decreased, among women with PCOS during a 21-year follow-up period. Only higher TG levels persisted among postmenopausal women with PCOS compared to the controls (31). Hudecova et al. (46) reported significant differences regarding glucose, triglycerides, HDL- cholesterol, and blood pressure between patients with PCOS and healthy controls. However, following adjustment for BMI, postmenopausal status, and hormone use with multivariate linear regression analyses, only the difference in triglycerides was found to persist among Swedish women with previous histories of PCOS (46). In summary, dyslipidemia persists throughout life in women with PCOS, together with a heightened risk of dyslipidemia linked to obesity.

Hypertension

Several studies have reported a greater prevalence of HT in women with PCOS (37,47). One recent meta-analysis showed a greater prevalence of HT among patients with PCOS compared to control populations. However, this was observed only at reproductive age and not among menopausal women with histories of PCOS during the reproductive period (47). The Dallas Heart study showed higher incidences of BMI and HT among women with PCOS (mean age: 40 years) compared to control women with regular cycles. This was also found to persist at age-, BMI-, and ethnicity-matched analysis (36). Pinola et al. (20) reported significant increases in both systolic blood pressure (SBP) and diastolic blood pressure in both PCOS populations compared with controls. In addition, higher rates of HT and higher BMI-adjusted SBP were found in hyperandrogenic women with PCOS aged over 39, although the mean values in both groups were within normal limits (20). Schmidt et al. (31) and Wild et al. (29) both observed higher prevalence of HT in women with PCOS, although other studies have reported no significant differences in the prevalence of HT between aging women with PCOS and the general population (30,38,42).

The risk of HT is generally higher among women with PCOS. However, further research is now needed to establish the risk of HT in aging women with PCOS beyond menopause.

Cardiovascular disease and risk factors

The menopausal transition is characterized by significant alterations in cardiovascular risk factors. These are associated with both chronological and ovarian aging. High circulating androgen levels have been linked to an unfavorable cardiovascular risk profile and a greater prevalence of subclinical atherosclerosis among women of postmenopausal age (10,48). Although many studies have shown a greater incidence of cardiometabolic risk factors among women with PCOS, there has been little evaluation of this association in older women with PCOS (10). Recent guidelines recommend that adolescents and women with PCOS be screened for CVD risk factors, such as a family history of early CVD, smoking, IGT/DM, HT, dyslipidemia, and abdominal adiposity (23,49). In addition to these familiar cardiovascular risk factors, a number of studies have also associated PCOS with an increased carotid artery intima media thickness (CIMT), decreased arterial flow-mediated dilation, and coronary artery calcium (CAC) elevation (38,50,51). In a study of 125 women with PCOS and 142 healthy controls, Talbott et al. (51) observed greater CIMT in patients with PCOS (0.78 mm) compared to control women aged 45 or more (0.70 mm). These authors also reported that the difference remained significant, even after adjustment for BMI (51). On the 20th year of the CARDIA study, higher mean internal CIMT and bulb mean CIMT values were observed among women with PCOS evaluated at 45 years of age than in healthy controls. These patients also exhibited a 2.7-fold greater probability of elevated CAC (aOR: 2.7, 95% CI 1.31-5.60) compared with the healthy controls (38). In contrast, research from the Dallas Heart study recently reported a similar prevalence of CAC scores >10 among women with PCOS who had both oligomenorrhea and HA (n=55), and women with PCOS according to the Rotterdam criteria (n=144) and healthy controls with normal ovulation (n=170), despite a higher prevalence of CVD risk factors among women with PCOS (36). No association was observed between presumed

PCOS and either greater CIMT or peripheral artery disease in the Rotterdam study (30). Meun et al. (28) recently investigated the cardiometabolic characteristics and prevalence of CVD in middle-aged patients with PCOS (mean age: 50.5 years) compared with age-matched controls. No evidence was found suggesting an increased 10-year cardiovascular risk or more serious atherosclerosis compared with control women from the general population (28).

Despite evidence supporting the idea of a greater risk of subclinical atherosclerosis in women with PCOS, the results of studies investigating the prevalence of CVD events are controversial, especially in post-menopausal women. A retrospective cohort analysis from the United Kingdom that spanned 20 years reported high incidences and age-specific prevalence of DM, myocardial infarction (MI) and angina among women with PCOS, with more than 25% of women with MI or angina being over 65 (39). However, no relationship was determined in the Rotterdam study between androgen elevation and incidence of stroke, coronary heart disease (CHD), or CVD (30). Another follow-up study of women with PCOS reported that CVD risk markers persisted into the postmenopausal period with no heightened incidence of stroke, CVD or mortality (31). No association with CHD or mortality was observed in a small cohort of postmenopausal PCOS patients exhibiting a trend toward more prevalent CHD, with multi (two or three) vessel disease being determined in 42% patients compared with 27% of women without clinical characteristics of PCOS (42). However, great caution must be employed when interpreting the findings of all such studies, particularly in the light of methodological and reporting limitations, incomplete diagnosis of PCOS, and the small sample sizes involved. Iftikhar et al. (52) observed no increase in CV events, including MI, coronary artery bypass graft surgery, death due to CV disease, and stroke, over 20-year follow-up. Wild et al. (29) reported that while a history of CHD was not significantly more frequent in women with PCOS, the crude OR for stroke was 2.8 (1.1±7.1). The incidence of stroke may be due to the longer follow-up period and older age. A recent study from the National Registry in Denmark reported a greater risk of CVD (HR: 1.3, 95% CI 1.2-1.4) in premenopausal patients with PCOS following adjustment for confounders, including obesity and DM. Obesity, DM, infertility, and previous use of oral contraceptive were associated with a heightened risk of development of CVD in these patients (53).

Studies and guidelines state that cardiometabolic risk factors are more prevalent among women with PCOS. Lifestyle management and modification are particularly recommended for primary CVD prevention, targeting dyslipidemia, and glucose abnormalities. Metformin and treatments for dyslipidemia should be added if necessary (44,49).

Cancer

Women with PCOS are exposed to risk factors, including null-parity, obesity, and prolonged unopposed estrogen, that are associated with EC. Barry et al. (54), reported a significantly greater risk of EC (OR: 2.79; 95% CI, 1.31-5.95, p=0.008) among women with PCOS, but that no significant change occurred in the risk of ovarian and breast cancers. However, once studies involving subjects aged over 54 years had been removed from the results, the risk for women with PCOS increased for EC and significantly for ovarian cancer, although no significant risk was observed for breast cancer (54). In addition, a cohort of 786 women with PCOS (mean age: 56.7 years) were followed-up for a mean 31 years (range: 15-57) following diagnosis of PCOS. The prevalence of EC was higher among women with PCOS than in the control subjects (2.2% vs 0.4%; p=0.001) (55). Based on The Danish National Patient Register data, Gottschau et al. (56) calculated an overall four-fold increased risk of EC, but found no relationship between PCOS and breast or ovarian cancer. In summary, although the small numbers of events involved represent a limitation in these studies, health professionals and women with PCOS should nevertheless be aware of a two- to six-fold increased risk of EC.

Conclusion

PCOS is a reproductive and metabolic disorder associated with a number of long-term health risks such as obesity, IGT, T2DM, HT, dyslipidemia, MS, cardiovascular risk factors, and EC during reproductive age, especially in patients possessing classic phenotypes. However, the question of whether the presence of PCOS results in a significant increase in such morbidity and mortality in older women with PCOS is still controversial. Although these cardiometabolic risk factors are more common among women with PCOS, currently there is no strong evidence for increased cardiovascular morbidity and mortality in aging women with PCOS.

An established, long-term history of OA and HA during the reproductive years in the peri-postmenopausal period may suggest a presumptive diagnosis of PCOS. The majority of studies concerning this topic have a number of limitations, including self-report diagnosis, being retrospective or cross-sectional in character, small sample sizes, inappropriate diagnostic criteria for PCOS without defining phenotypes, and limited follow-up. Extensive prospective cohort studies in which healthy controls in addition to patients with defined PCOS phenotypes are observed and monitored from the early reproductive period into the late postmenopausal period should now be performed in order to clarify morbidities and mortality in older women with PCOS.

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False-positive results of lupus anticoagulant tests should be kept in mind in pregnant patients receiving low molecular weight heparin

To the Editor,

We read the article by Dr Izhar et al. (1), entitled "Anti-phospholipid antibodies in women presenting with preterm delivery because of preeclampsia or placental insufficiency", in the last issue of your journal with great interest. The authors observed a high prevalence of anti-phospholipid antibodies (APLA) in women who have preterm delivery due to preeclampsia or placental insufficiency (PREPI), corroborating the results of previous reports. Their findings are of great interest and finally shed some more light on this interesting topic. Therefore, we would like to commend the authors for addressing this issue. However, several points caught our attention while reading this paper and we would like to highlight these to the reader.

Firstly, as already mentioned by the authors, classification criteria for anti-phospholipid syndrome (APS) includes both clinical and laboratory criteria. The clinical criteria consist of vascular thrombosis and/or pregnancy morbidity. Although the association of APLA with preeclampsia was discussed extensively by the authors, no information was presented regarding the cases in which vascular thrombosis was present. Vascular thrombosis in APS can affect any vascular bed, including venous, microvascular and arterial vessels and can complicate pregnancy (2). Hence it would be beneficial for the authors to perform a subgroup analysis assessing APLA levels in pregnant patients with a history of arterial or venous thrombosis.

Secondly, and more importantly, we think that the authors should have indicated and discussed whether the use of low molecular weight heparin (LMWH) or other anticoagulants had interfered with lupus anticoagulant (LAC) testing in their patient group. Although the detection of LAC according to the guidelines of the International Society on Thrombosis and Hemostasis criteria include screening, mixing and confirmation tests, measured on two or more occasions at least 12 weeks apart, is strictly reliable, both false-positive and false-negative results have been described in literature due to use of heparin or LMWH (3-5). In this context, Martinuzzo et al. (5) study is important for demonstrating an increased rate of false-positive LAC test results in plasma of patients with previous negative LAC tests that receive enoxaparin 40 mg/day. Furthermore, enoxaparin has been shown to affect tests for LAC not only in screening and mixing, but also in confirmatory studies. In accordance with these findings, the Scientific and Standardization Committee for LAC/anti-phospholipid antibodies suggest that anticoagulation with any drug, including unfractionated heparin, LMWH and direct oral anticoagulants, may potentially complicate LAC detection, simply because anticoagulants usually lengthen test clotting times (i.e., the activated partial thromboplastin time and dilute Russell's viper venom time), currently proposed for LAC detection (4,6). Therefore, we think that it would have been advisable for the authors to have mentioned the possible effect of LMWH on positive LAC test results in their patient group. Moreover, further analysis of repeated LAC tests after discontinuation of LMWH in patients who were using LMWH at the time of initial positive LAC should have been included in the article.

In conclusion, we fully appreciate the finding that APLA has a significant effect on preterm delivery due to PREPI. Thus, we suggest that anti-FXa activity should also be measured in patients who are known to be on LMWH treatment and if the activity is within the therapeutic range, LAC testing can be carried out if reagents contain heparin neutralizers.

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Author's Response

Dear Editor,

We would like to thank the reader for critically reading and evaluating our article. We would like to clarify to the readers that none of our women had any history of thrombosis or known thrombophilia, so a sub-analysis of such cases was not required. Moreover, we used the standard guideline criteria for detecting LAC, which they also agree is strictly reliable. They have quoted that prolongation of clotting time with use of anticoagulants "can potentially" affect results. We need to take the findings of these studies with a grain of salt. The authors have referred to studies that show anticoagulant use complicates detection of LAC. We tested subjects for all three antibodies and we tested them as per the standard criteria, which still remains the most stringent and widely accepted criteria for identifying anti-phospholipid antibody syndrome. Until a firm evidence base is there, we cannot negate or affirm that detection is altered. However, we would agree that few reports exist that are in agreement with complications in detecting LAC.

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Two port laparoscopic trachelectomy without the use of ureteral stents

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Abstract

Trachelectomy is a notoriously difficult laparoscopic procedure, often because of remaining scar tissue from a prior supracervical hysterectomy, as well as the necessity to clear vital organs, including the bladder and the rectum, out of the plane of dissection in order to remove the cervix. Many authors have suggested techniques involving ureteral stents to minimize the chance of ureteral injury. Our institute presents this two-port laparoscopic technique without the use of stents, which we believe safely accomplishes the trachelectomy through very minimally invasive means.

Keywords: Trachelectomy, laparoscopy, single port, two port, robotic

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Introduction

Trachelectomy is a notoriously difficult laparoscopic procedure (1). The reasons for this include remaining scar tissue from a prior supracervical hysterectomy, as well as the necessity to clear vital organs, including the bladder and the rectum out of the plane of dissection in order to remove the cervix (2,3). Based on our review of the literature, many authors have discussed the use of novel techniques (4,5), ureteral stents (6,7), and uterine manipulators (5) for the purpose of performing trachelectomy (8). In this video we present our technique for laparoscopic, two-port trachelectomy, using a novel approach of vaginal tension on the cervix to complete the colpotomy.

Objective

To demonstrate a technique to perform a laparoscopic trachelectomy in the safest, most minimally invasive, cost effective way possible, without the use of ureteral stents (Video 1). We designed a surgical technique including several novel aspects. First, we began dissection on the cervical stump with a linear horizontal incision to maximize the distance from the bladder and rectum (Figure 1). Next, we used a technique of maintaining pressure against the vaginal cuff, deep within the abdomen, to move the ureters laterally, thus eliminating the need for ureteral stents. We overcame the obvious problem of keeping the cervix planted against the manipulator by the novel usage of a laparoscopic tenaculum, used to hold the cervix from the vaginal approach through the manipulator (Figure 2). Thus we were able to complete the circumferential colpotomy (Figure 3) with the cervix firmly held against the internal ring of the manipulator at all times.

This manuscript has been reviewed by the Institutional IRB Board at Marchand Institute and was found to be exempt from IRB review (July 2018).

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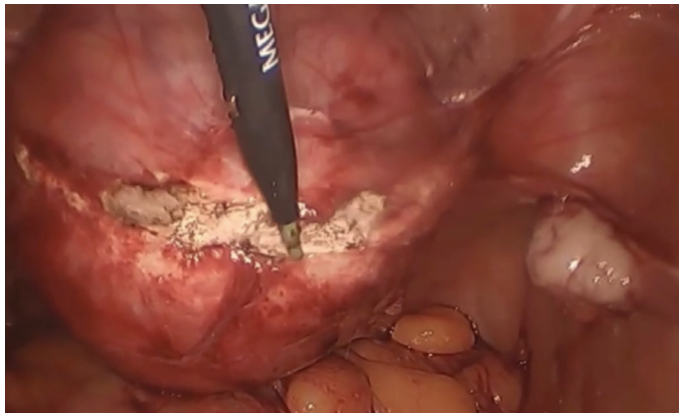

Figure 1. Initial dissection into the cervical stump is started in a linear pattern in order to maximize the distance from both the bladder and the rectum

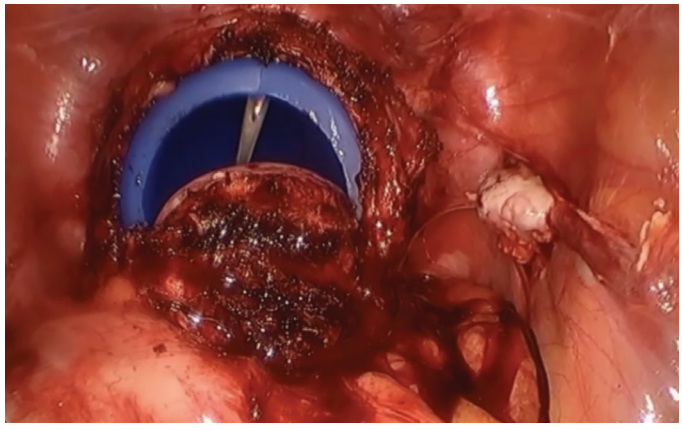

Figure 2. A 5 mm laparoscopic, sharp-tooth tenaculum is inserted vaginally in order to grasp the cervix and hold tension against the manipulator. This allows the manipulator to be pushed cephalad while completing the colpotomy. The resulting force pushed the ureters laterally, minimizing the risk of ureteral injury

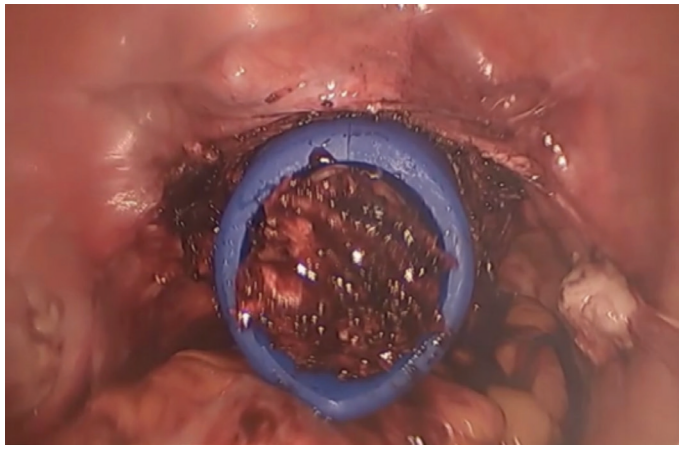

Figure 3. The colpotomy was completed and the cervix is free within the manipulator

Design

A narrated video demonstration of the surgical procedure (Canadian Task Force Classification III).

Setting

The setting was a suburban hospital in the United States.

Interventions

The patient was an obese, 46-year-old female with pain in the area of the cervix and vaginal bleeding 10 years after open supra-cervical hysterectomy. Two-port laparoscopic trachelectomy without ureteral stents was performed. Our novel technique was successful in completing the procedure without complications. We have explained the technique and instrumentation in this video, for reproducibility. The patient was discharged 26 hours after surgery and her recovery was uneventful.

Conclusion

This technique is a feasible, reproducible procedure for laparoscopic trachelectomy. Novel aspects of this technique may effectively eliminate the need for pre-operative ureteral stents in some cases.

Acknowledgement: *The Marchand Institute for Minimally Invasive Surgery would like to acknowledge the efforts of all of the students, researchers, residents and fellows at the institute who put their time and effort into these projects without compensation, only for the betterment of women's health. We firmly assure them that the future of medicine belongs to them.*

Video 1. Fully narrated video demonstration of our described technique of two port laparoscopic trachelectomy without the need for ureteral stents

https://www.doi.org/10.4274/jtgga.galenos.2020.2020.0027.video1

Commitment to Diversity: *The Marchand Institute remains committed to diversity and tolerance in its research, and actively maintains a workplace free of racism and sexism. Greater than half of the authors for this study are female and many represent diverse backgrounds and under-represented ethnic groups.*

Informed Consent: *Patient gave written consent for usage of video prior to and after procedure.*

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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Radical vulvectomy with right gluteal and left medial thigh V-Y advancement flap reconstruction

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Abstract

Vulvar cancer is rare. The vulva constitutes the external female genitalia and it is associated with the perineum with the intersection of urinary, sexual and anal systems. The deep anatomy of the perineum in the urogenital and anogenital triangle should be well-known to gynecological oncologists. Radical vulvectomy is the surgical treatment of choice in gross tumors expanding over the vulvar skin. After this type of excision, reconstruction is critically important because it is not always feasible to suture the vulvar defect in a primary manner. Thus, the reconstruction options should also be known to gynecological oncologists. Here, we present a video of radical vulvar cancer surgery, which was performed on a cadaver with gluteal and medial thigh V-Y advancement flap reconstruction.

Keywords: Vulvar cancer, flap, vulvectomy, perineum, cadaveric

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Introduction

Vulvar cancer is the least common gynecological malignancy and makes up around 2-5% of gynecological cancers. It usually presents with pruritus and sometimes with a vulvar lesion, which is commonly detected on the labia majora. Squamous cell carcinoma is the most common histological type, constituting approximately 90%, and management does not significantly change among the subtypes. Vulvar cancer is generally diagnosed in the early stages and surgical removal of the vulva with a conservative or radical approach forms the cornerstone of treatment, especially for early-stage disease (1,2). Inguinal lymph nodes are the site of lymphatic dissemination and lymph node involvement is one of the most important prognostic factors associated with stage, adjuvant treatment and survival. Thus inguino-femoral lymphadenectomy is a part of surgical treatment (3).

Due to the small number of cases, the learning curve for gynecological oncology fellows concerning vulvar cancer surgery is steep. Additionally, after removal of the vulva the reconstruction phase is not always feasible with primary suturation. Hence, plastic surgeons will apply vulvar flap replacement and this is not always performed by all gynecological oncologists. Currently, wide local vulvar excision on the side affected by the tumor, with similar margins to radical vulvectomy, is the main type of surgery in early-stage vulvar cancer. In contrast, in gross tumors expanding across the vulvar skin, radical vulvectomy is the choice of surgical treatment. In this video article we demonstrate radical vulvar cancer surgery

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that was performed using a cadaver during the Vulvar Cancer Surgery Cadaveric Workshop; an International and European Society of Gynaecological Oncology endorsed meeting held on the 31th August 2018 at Bahçeşehir University Faculty of Medicine, Department of Anatomy, İstanbul, Turkey.

Vulvar anatomy

Vulva is the term used to describe the entire external female genitalia. The vulva is comprised of the mons pubis, labia majora, labia minora, clitoris, vaginal vestibule and vestibular glands. The anastomotic vessel arc of the external and internal pudendal arteries supplies the vulvar region. The innervation of the vulva is provided by the ilioinguinal nerve, genitofemoral nerve and pudendal nerve (dorsal clitoral nerve and perineal nerves). Superficial inguinal lymph nodes are the primary site of lymphatic drainage of vulva and after the superficial lymph nodes, the drainage flows over the cribriform fascia to the deep femoral lymph nodes (4).

The perineum is the region between the anus and the upper portion of the clitoris, at the mons pubis. The boundaries of the perineum are; anteriorly pubic symphysis and arcuate ligament of the pubis, posteriorly coccyx, anterolaterally ichiopubic rami and ichial tuberosities, posterolaterally sacrotuberous ligament, superiorly pelvic floor and inferiorly the skin. Superficially the skin covers the perineum while the pelvic diaphragm and the levator ani muscle forms the deepest part (5). A line between the ischial tuberosities divides this diamond-shaped region into the anteriorly located urogenital and posteriorly located anal triangle (Table 1).

Radical vulvectomy with right gluteal and left medial thigh V-Y advancement flap reconstruction: surgical technique (Video attachment shows the surgical technique)

1. The circumferential outer incision on the vulvar skin aims to excise the tumor with clear margins. A pathological margin of 0.8 cm is critical after the tissue shrinkage with formalin. Thus, an incision 2 cm laterally, from the tumor will be optimal for clear margins. The resection margin will decrease to 1 cm around the urethra and anus to protect the functions of these structures. Nevertheless, the distal urethra (1 cm) could be sacrificed without any harm to function. In some cases, partial external anal sphincter excision may also be applied (6).

2. The incision deepens down to the subcutaneous fatty tissue and afterwards down to the inferior fascia of urogenital diaphragm which is termed as perineal membrane, by the way the contents of the superficial perineal space (ischiocavernosus, bulbospongiosus and superficial transverse perineal muscle) are excised with the vulvar specimen. The arterial supply from the internal pudendal artery come from the 5 and 7 o'clock directions and they should be ligated or sutured.

3. The upper part of the vulvar incision deepens down to the pubic periosteum, which is medial to the adductor fascia. Here, the suspensory ligament of the clitoris should be ligated or sutured.

Table 1. Layers of the perineum from inferior to superior (from skin to pelvic floor)

A. Urogenital triangle
Skin
Superficial perineal fascia
Superficial fatty layer (Camper's fascia)
Membranous layer (Scarpa's fascia)
Deep perineal fascia
- This fascia covers the superficial perineal muscles located at the superficial perineal pouch - **Superficial perineal pouch** - the lateral border is formed by the ischiopubic rami
Bulbospongiosus muscle
Ischiocavernosus muscle
Superficial transverse perineal muscle
Perineal branch of pudendal nerve
Crura of clitoris
Bulbs of vestibule
Perineal membrane (Inferior fascia of urogenital diaphragm)
- The perineal body is continuous with the perineal membrane - **Deep perineal pouch** - the lateral boundary is formed by the inferior portion of the obturator internus muscle
Deep transverse perineal muscle
External urethral sphincter
Proximal urethra
Internal pudendal vessels
Dorsal nerve of clitoris
Superior fascia of urogenital diaphragm
Pelvic floor
Levator ani muscle
Coccygeus muscle
B. Anal triangle
Anal canal, sphincters, the ischio-anal fossa, nerves and vessels are the contents of the anal triangle
Skin
Superficial fascia
Superficial fascia of anal triangle contains the subcutaneous fatty tissue
Deep fascia
Deep fascia of the anal triangle is inferior to the levator ani muscle and covers the ischioanal fossa and its lateral part
Pelvic floor
Levator ani muscle
Coccygeus muscle

4. At the inferior part of the incision, dissection is performed over the rectovaginal septum.
5. A circumferential inner incision encircling the urethra and vaginal introitus is performed.
6. The outer incision on the labia majora and skin is combined with the inner incision, which is around the vaginal introitus, and the vulvar specimen is excised totally (Figure 1).
7. The wound is closed primarily in most cases. Deep structures are sutured with 2-0 delayed absorbable materials to prevent any dead space. Skin should be closed in a tension-free fashion.
8. If a tension-free closure is not possible, the vulvar defect should be closed by a flap in advance with an adequate blood supply, which may prevent later cosmetic and functional problems. For flap replacement the gluteal or the medial thigh is incised in a manner of "V-Y" down to the level of the muscle fascia (Figure 2). During this step, the perforators arising from the internal pudendal artery are secured, and electrocautery could be used in most of the circumstances. When the flap is mobilized in all directions, it is advanced medially to the vaginal inner wall in a tension-free manner. The "dog ear" formation, which is shaped at the edges, is removed and the flap is sutured to the surrounding tissue with the aim of closing all the layers (7) (Figure 3). Always consider a multidisciplinary approach for reconstruction of the vulvar defect (8).

Complications of radical vulvectomy

Since the surgery is extremely radical and impinges on the urinary, sexual and anal organs, there may be many dysfunctions related to these systems (9).

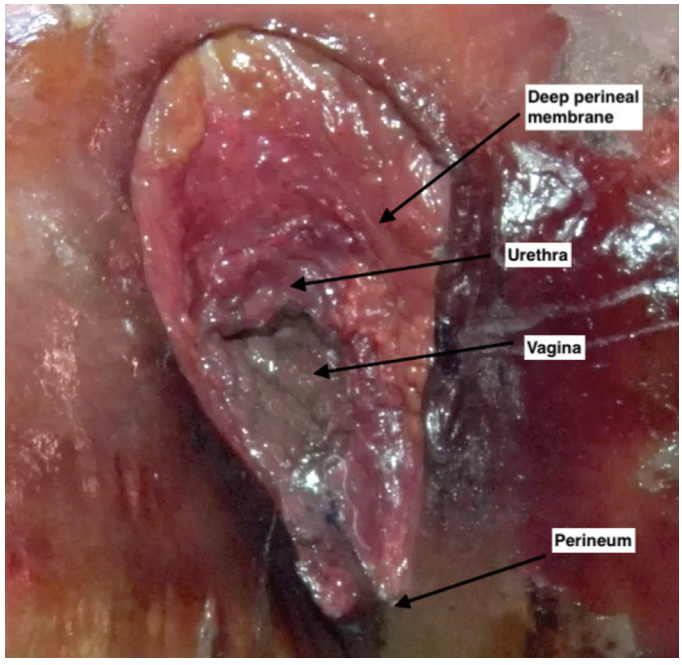

Figure 1. The vulvar region after excision of the radical vulvectomy specimen

- Wound complications

Wound breakdown and wound infection are particularly prevalent in obese patients at the end of the first week. Suction drains are suggested in selected patient groups.

- Flap complications

They mostly arise due to inappropriate vascular supply or increased tension.

- Urinary complications

Infection and involuntary urine loss are the probable complications with regard to the radicality of the surgery.

- Psychosocial and sexual dysfunction

It is an important issue that may be revealed and mitigated with professional support.

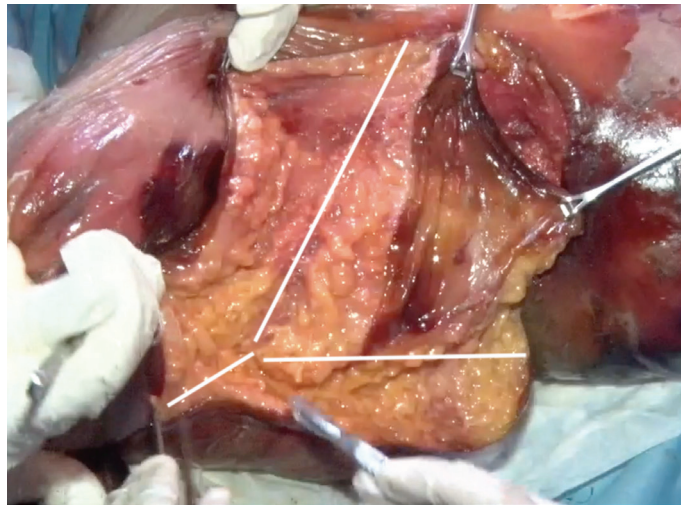

Figure 2. The incision for left gluteal V-Y flap advancement to the level of muscle fascia

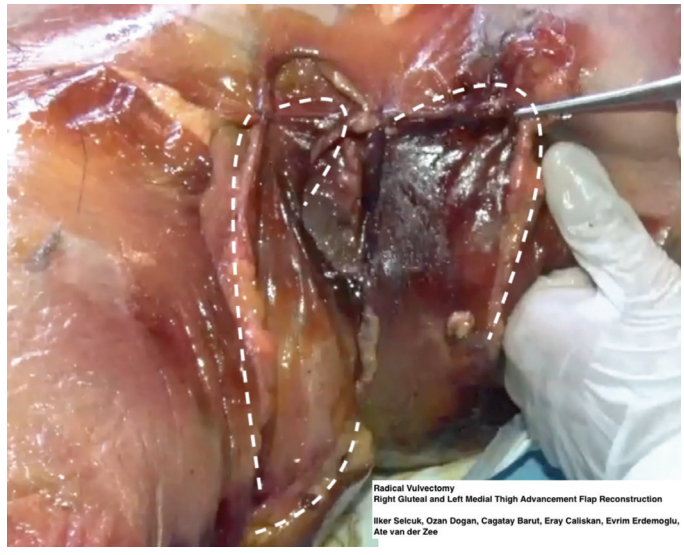

Figure 3. Vulvar reconstruction with V-Y advancement flap and closure of deep and superficial layers

Conclusion

Vulvar cancer is rare and curative surgery is a radical procedure. The anatomy and the reconstruction techniques should be known by all gynecological oncologists in order to achieve optimal surgical outcomes more widely.

Video 1.

https://www.doi.org/10.4274/jtgga.galenos.2020.2020.0055.video1

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Laparoscopic view of endosalpingiosis in a woman with dermoid cyst and endometriosis

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Abstract

Endosalpingiosis is, like endometriosis, the presence of cystic masses outside of the salpinx which contains fallopian tube epithelium. Endosalpingiosis can be seen on the surface of ovaries, tubal serosa, uterine serosa, myometrium, and also in the bladder. The main clinical features of endosalpingiosis are pelvic pain, adnexal mass which mimics cancer, and urinary symptoms. Herein, we present a surgical video of endosalpingiosis in a woman with endometriosis and a dermoid cyst.

Keywords: Endosalpingiosis, laparoscopy, endometriosis, dermoid cyst

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Introduction

Endosalpingiosis is, like endometriosis, the presence of cystic masses outside of the salpinx, which contains fallopian tube epithelium with affected structures that may include the ovarian cortex, uterine serosa, and the surface of other pelvic organs, and the inguinal region (1-5). Endosalpingiosis is usually an incidental finding at the time of surgery. Although endosalpingiosis is a benign and rare condition, it can mimic peritoneal cancer or metastases (6). Experienced pathologists are crucial for exact diagnosis. Endosalpingiosis differs histologically from endometriosis since it has ciliated glandular epithelium, no endometrium-like tissue, and does not display the same inflammatory reactions. Endosalpingiotic glands should be discriminated from mesonephric remnants in the pelvis, which are common incidental microscopic findings in the region of the fallopian tube. Mesonephric remnants are typically located more deeply than endosalpingiosis and characteristically have a collar of smooth muscle under the epithelial lining, which is typically a single layer of non-ciliated, low columnar to cuboidal cells. As in the present case, the endosalpingiotic tissue contains columnar and ciliated epithelium with intercalated cells, which possess a clear cytoplasm (7,8). Thus it is important to raise awareness of endosalpingiosis, but radical surgery should be limited, due to high recurrence rates.

The purpose of this video article (Video 1) was to demonstrate a laparoscopic view of incidental endosalpingiosis, concomitant with dermoid cyst and endometriosis. This operation was recorded at a university hospital. A 40-year-old woman was admitted to our outpatient clinic due to pelvic pain with a duration of six months. Her medical history included cesarean section and laparoscopic ovarian cyst surgery. Transvaginal ultrasonography revealed a 5 cm dermoid cyst in the right adnexal area. Tumor markers and other biochemical parameters were within the normal range. In light of the findings, laparoscopic surgery was proposed. A 10 mm trocar was inserted through the umbilicus for the optic system and three ancillary ports were also employed. Endoscopic visualization revealed the right

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ovary with a 5 cm cyst, diffuse clear cystic masses involving the uterine serosa and an endometriotic lesion on the vesico-uterine peritoneal fold (Figure 1, 2). Left ovary and other organs were of normal appearence. The cyst wall was cauterized with a bipolar instrument. During dissection via laparoscopic scissors, the cyst was punctured. Cyst content was aspirated, immediately. Then, the dermoid cyst wall was extirpated with a traction counter-traction technique. The cyst wall was placed in a surgical sterile surgical glove and removed via a 10 mm optic port. The pelvic peritoneal cavity was thoroughly washed with sterile saline. Small bleeds were cauterized with the bipolar instrument and then the right ovary was sutured. A punch biopsy was taken from the clear cysts on the uterus. After the coagulation of the endometriotic lesions on the pelvis, the operation was terminated (Figure 3). Histopathological diagnosis of the punch biopsy material from the clear cysts on the uterus was reported as endosalpingiosis.

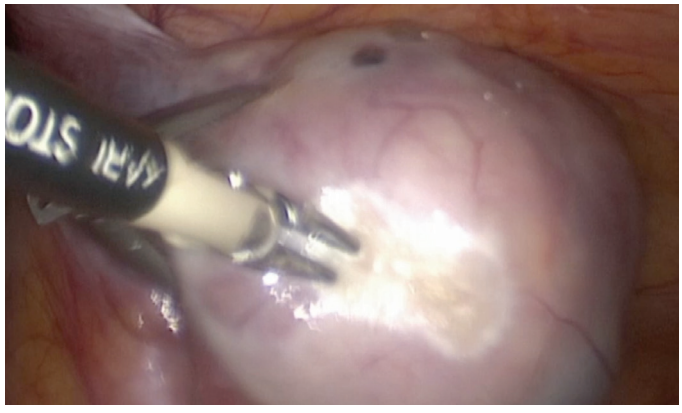

Figure 1. 5 cm diameter dermoid cyst in the right ovary

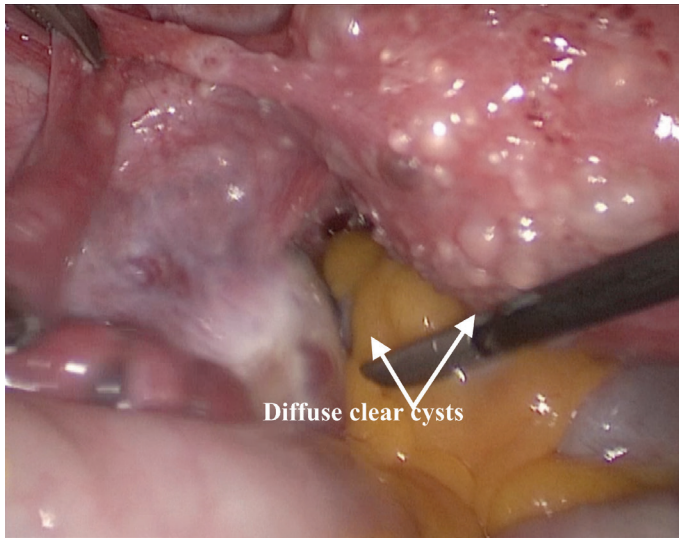

Figure 2. Diffuse clear cysts on the surface of the uterus, fallopian tube, and the left ovary

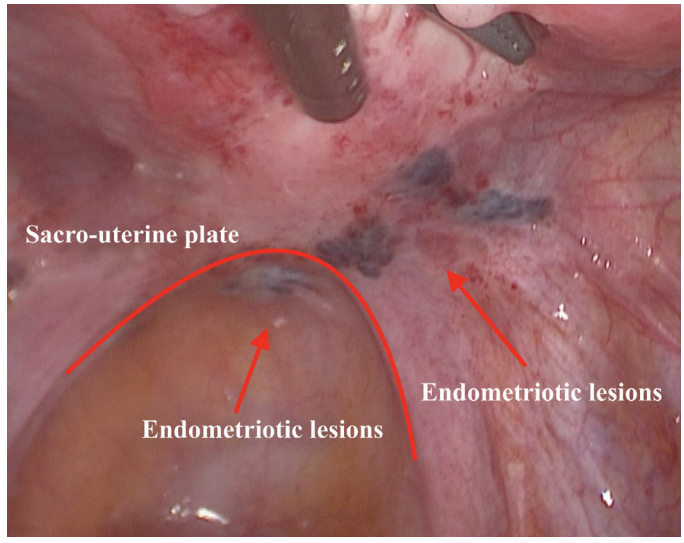

Figure 3. Endometriotic lesions on the peritoneum of the pouch of Douglas

Video 1. Stepwise demonstration of the operation with narrated video footage

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Informed Consent: *Informed consent was obtained from the patient.*

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Acknowledgements for the Year 2021
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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.medical.theconferencewebsite.com/conferences/obstetrics-and-gynaecology)

January 02-08, 2022	37th Annual Conference of Obstetrics, Gynecology, Perinatal Medicine, Neonatology and the Law, Orlando, FL, United States
January 31-February 05, 2022	Society for Maternal-Fetal Medicine (SMFM) 42nd Annual Pregnancy Meeting, Kissimmee, FL, United States
March 03-06, 2022	International Society for the Study of Womens Sexual Health Annual Meeting, Dallas, TX, United States
March 15-19, 2022	Society for Reproductive Investigation (SRI) 69th Annual Scientific Meeting, Denver, CO, United States
March 18-21, 2022	Society of Gynecologic Oncology (SGO) Annual Meeting, Phoenix, AZ, United States
March 31-April 03, 2022	ASCCP 2022 Scientific Meeting on Anogenital & HPV-Related Diseases, San Diego, CA, United States
April 03-06, 2022	International Federation of Fertility Societies (IFFS) 24th World Congress, Athens, Greece
May 06-09, 2022	American College of Obstetricians and Gynecologists (ACOG) 2022 Annual Clinical and Scientific Meeting, San Diego, CA, United States
May 11-14, 2022	International Society of Gynecological Endocrinology 20th World Congress, Florence, Italy
May 18-21, 2022	8th Congress of the Society of Endometriosis and Uterine Disorders, Athens, Greece
May 26-29, 2022	16th ISUOG International Symposium, Cairo, Egypt
May 28-June 01, 2022	**XIV. TURKISH GERMAN GYNECOLOGIC CONGRESS, Antalya, Turkey**
June 29-July 02, 2022	XXVIII European Congress of Perinatal Medicine (ECPM), Lisbon, Portugal
July 03-06, 2022	European Society of Human Reproduction and Embryology (ESHRE) 38th Annual Meeting, Milan, Italy
September 16-18, 2022	32nd World Congress on Ultrasound in Obstetrics and Gynecology, Venue not announced yet
September 30-October 02, 2022	International Gynecologic Cancer Society (IGCS) 2022, Meeting, New York, NY, United States
October 02-05, 2022	ESGE 31st Annual Congress, Lisbon, Portugal
October 22-26, 2020	American Society for Reproductive Medicine (ASRM) 78th Annual Meeting, Anaheim, CA, United States
October 26-29, 2022	18th World Congress on Menopause, Lisbon, Portugal
November 24-26, 2022	The 30th World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Amsterdam, The Netherlands
November 30-December 04, 2022	The 51st American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Denver, CO, United States

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.kongre2020.com)

March 10-13, 2022	16. Uludağ Jinekoloji ve Obstetrik Kış Kongresi, Bursa, Türkiye
March 24-27, 2022	CİSED 6. Ulusal Kongresi, Antalya, Türkiye
May 19-22, 2022	Türk Jinekoloji ve Obstetrik Derneği 2022, Antalya, Türkiye
May 28-June 01, 2022	**TAJEV - 14. TÜRK- ALMAN JİNEKOLOJİ KONGRESİ, Antalya, Türkiye**
September 08-11, 2022	3. Uluslararası KKTC Obstetri ve Jinekoloji Kongresi, Girne, KKTC
September 22-25, 2022	4. Obstetrik ve Jinekoloji Tartışmalı Konular Kongresi, Antalya, Türkiye
November 03-06, 2022	Uluslararası İzmir Jinekoloji ve Obstetri Kongresi, Muğla, Türkiye