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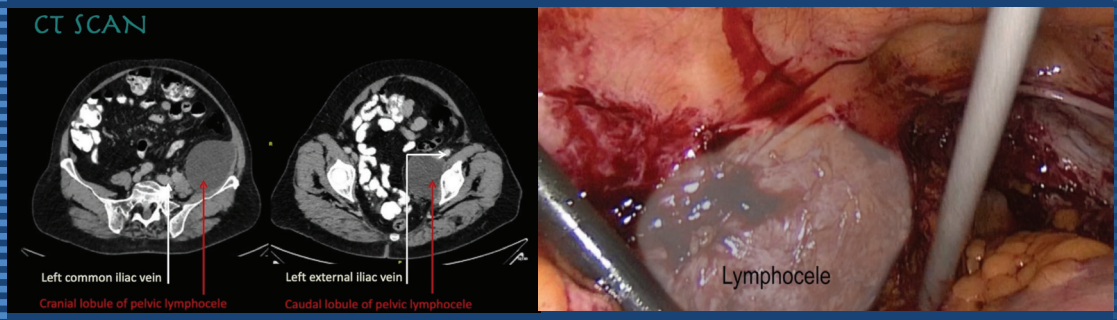

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Aims and Scope

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.

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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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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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Ertan AK, Tanriverdi HA, Schmidt W. Doppler Sonography in Obstetrics. In: Kurjak A, Chervenak FA, editors. Ian Donald School Textbook of Ultrasound in Obstetrics and Gynecology. New Delhi, India: Jaypee Brothers; 2003. p. 395-421.

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the first issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2022. This issue is consisted of seven articles and one review that we hope you will read with interest. Here we share some of our favorite articles that were published in this issue of the journal.

There are conflicting studies about the effect of platelet-rich plasma (PRP) applications on success in in-vitro fertilization (IVF) treatment. You will read an article evaluating the effects of intra-ovarian PRP injections on IVF outcomes of poor responder women and women with premature ovarian insufficiency. You will also read an interesting study which assessed the optimal number of follicular flushes on retrieval rate and quality of oocytes in mono-follicular IVF cycles. You will get the occasion to read a systematic review and meta-analysis assessing the efficacy of hyoscine for the management of pain during in-office hysteroscopy procedures.

I would also like to invite you to join us for 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 with a rich scientific program and pre-congress courses as well as joint sessions with international societies. At this year's congress we will be having lectures with the world's most reputable speakers; Prof. Gunter Noe (Laparoscopic pelvic floor surgery: a holistic approach on native tissue repair), Prof. Ceanea Nezhat (Adolescent endometriosis: A call to action on early detection), Prof. Kutluk Oktay (Fertility preservation for Turner syndrome), Prof. Wolfgang Holzgreve (Stem cells in obstetrics and gynecology).

Dear Researchers,

Our congress will reward the best 3 abstracts. The purpose of this reward is to show our colleagues our appreciation for their productivity and also motivate our young colleagues for the forthcoming years. Also the best video presentation which will be elected by the Scientific Committee will receive a 5.000 TL "Dr. Aysun - Cihat Ünlü Special Reward".

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Editorial

Please do not forget to mark the congress on your calendars in order to not to miss this scientific festival. I would like to wish you a happy and healthy spring 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

Remote assessment and reinforcement of patient awareness of role of lifestyle modification and treatment adherence in polycystic ovary syndrome using an online video based educational module

Aniket Gour, Pankhuri Dubey, Archana Goel, Ajay Halder
Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Bhopal, India

Abstract

Objective: To evaluate the role of an online, video-based, structured, educational module in increasing awareness in women with polycystic ovary syndrome (PCOS).

Material and Methods: Patients with PCOS were assessed for baseline awareness about PCOS, quantified as "awareness score", using a validated questionnaire. Topics assessed included factual and conceptual knowledge of the disease and awareness of behaviour-related lifestyle modification and therapy compliance in PCOS. An educational video module was shown to the participants which covered normal menstrual physiology, symptomatology, pathophysiology and natural history of PCOS, a comparative animation of healthy versus unhealthy lifestyle, indications of pharmacological intervention, and role of treatment adherence. The questionnaire was re-administered after exposure to the educational module, and effectiveness of the teaching method was evaluated by comparing pre and post test scores.

Results: The total number of subjects was 41. Baseline knowledge was "fair" in 17.1%, "moderate" in 48.8% and "good" in 34.1%. Significant increase in awareness scores was noted among participants regarding PCOS after exposure to the learning module from 15.09±4.31 to 18.60±3.85 ($p<0.00001$) with a large effect size (Cohen's $d=0.85$). Most (48.8%) of the respondents had baseline awareness in the "moderate" range (scores between; 11-17) whereas post intervention scores improved to the "good" category for 63.4% of the women.

Conclusion: The educational module was effective in significantly increasing knowledge about PCOS. Patient education is likely to help reinforce the message about lifestyle modification and continued compliance and may aid in promoting a patient-driven healthcare model in PCOS. (J Turk Ger Gynecol Assoc 2022; 23: 1-7)

Keywords: PCOS, knowledge, awareness, learning module, healthcare

Received: 30 September, 2021 **Accepted:** 23 December, 2021

Introduction

Traditional health care delivery has been evolving into a more collaborative and co-care model, wherein the patient is expected to take more responsibility for self-care and to actively participate in clinical decision making (1). Success of such a patient-centred health care model relies on the active engagement of patients as autonomous individuals who understand the full bio-psychosocial picture of their diagnosis, from their present physical and emotional needs to future health risks. Equipping women with accurate information tailored to their present condition is important as it improves their participation in treatment planning and breaks a perpetuating cycle of misinformation and poor health outcomes. This is especially instrumental in the treatment of chronic disorders, such as polycystic ovary syndrome (PCOS), where the lifestyle interventions are central to

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management, and therefore patient education and motivation for behavioural change as a part of initial therapy, right at the time of diagnosis, is crucial.

PCOS is a common disorder affecting 6-15% of women of reproductive age and is associated with risk of multisystem comorbidities, such as obesity, infertility, diabetes mellitus, dyslipidemia, hypertension, sleep apnea, future risk of endometrial cancer, depression, and impaired quality of life (2-6). Receiving a diagnosis of PCOS represents an opportunity to motivate women to take sustainable steps towards prevention of complications, yet the provision of relevant information by the health care providers about the role of lifestyle management and medical therapy on potential long-term consequences of PCOS is not satisfactory (7,8).

A typical patient with PCOS is an adolescent or a young woman. In addition, there may be a lack of reproductive health education in school, as comprehensive sexual and reproductive health education is not a part of the curriculum in most schools around the world. Further, unsatisfactory experiences of medical consultation that do not address their gaps in knowledge may leave women with an unmet need for information and affect their subsequent engagement with PCOS management and care (9).

Routine consultation needs to go beyond unidirectional, passive guidance on symptom treatment alone. Introducing structured educational elements during consultation in the primary health care setting is therefore important to help acquaint women with their disease and associated comorbidities. One method for patient education, a structured video-based educational module, was tested in the present study to assess its effectiveness in raising awareness about PCOS. The feasibility of introducing such components of patient education as a part of routine consultation is discussed.

Material and Methods

A longitudinal study using one group pretest-posttest design was conducted as a student project, funded by the Indian Council for Medical Research after approval from the All India Institute of Medical Sciences Bhopal Madhya Pradesh India, Institutional Human Ethics Committee and Review Board (approval number: IHEC-LOP/2018/STS0151). Consenting patients with a diagnosis of PCOS were selected to participate using non-probability purposive sampling.

Women aged 18-35 years, diagnosed with PCOS by the Rotterdam criteria (5,6) were included. The Rotterdam criteria, proposed by the group of experts in 2003, require two of three criteria to be met to fit the definition of PCOS: chronic anovulation, clinical and/or biochemical evidence of hyperandrogenism, and polycystic ovaries. Women with menstrual irregularity not fitting the diagnostic criteria, or those with PCOS complicated by chronic medical or surgical conditions were excluded. “Intervention” in the study was structured teaching using a video based educational module. Knowledge was compared before and after exposure to the video. The components of the study are described.

a) Pre-exposure (pre-test) component: Eligible and consenting participants were tested for relevant baseline knowledge using a validated questionnaire delivered via email or other social media platforms.

b) Exposure to educational module (intervention): An educational video (learning module) was shared via email or the preferred social-media platform after completion of the pre-test questionnaire. The module can be assessed at https://youtu.be/uxw5X6q4494. The contents of the video are described in Table 1.

c) Post-exposure (post-test) component: The questionnaire was readministered at the end of the video.

The questionnaire for the present study was prepared with a framework to test the knowledge and behavioural attitudes

Table 1. Components of the educational video

Module	Segment 1	Segment 2	Segment 3
Title	Normal menstrual cycle and pathophysiology of PCOS	Importance of lifestyle modification in PCOS	Pharmacological intervention and treatment adherence
Content	A. Video explaining about normal menstrual cycle comprising i. Length of the menstrual cycle, ii. Regularity of cycle, iii. Duration of bleeding & amount of flow. B. An animation explaining pathophysiology of PCOS	Comparative animation regarding healthy and non-healthy lifestyle including a description of role of diet, food supplements, daily physical activity, concept of calorie balance and body mass index, etc.	Animated expert interview regarding indication of pharmacokinetic interventions, their types and importance of treatment adherence. 1. Various treatment options, 2. Annoyances during period, 3. Effect on period after stopping medications, 4. Subfertility & complications during pregnancy, 5. Comorbidities and future health risks associated with PCOS including diabetes, obesity, endometrial cancer etc.

PCOS: Polycystic ovary syndrome

of participants with PCOS, before and after exposure to the educational module. It consisted of objective-style test content (a combination format of alternate response and close-ended multiple choice questions) consisting of a total of 25 individual questions. Subject matter for questions included, but was not limited to, factual and conceptual knowledge about the normal menstrual cycle, pathophysiology and natural history of PCOS, and behaviour-related awareness about lifestyle modification and treatment adherence in PCOS. An expert group, consisting of three senior gynaecologists, provided input on clarity, simplicity, and relevance of the content. A pilot test was carried out on a small sample of 20 respondents before being tested on the study population and internal consistency of the questionnaire was tested. The expert group reviewed the tool after pilot testing and provided recommendations on content validity which were incorporated to the revise the questionnaire. The list of questions is described in Table 2.

Table 2. Components of the questionnaire

Q1. Polycystic ovary syndrome or PCOS, a complex metabolic disorder, is diagnosed using Rotterdam criteria. Which of the following is true regarding diagnosis of PCOS with this criterion?	a. Delayed menstruation/irregular menstruation, b. Clinical evidence of excess male hormone (excessive dark coarse hair growth, acne etc), c. Ultrasonography showing enlarged ovaries or multiple small follicles (cysts) in ovaries, d. Any 2 of the above.
Q2. Which organ bleeds during menstruation?	a. Uterus b. Fallopian tube c. Ovary d. All the above
Q3. Which organ produces egg for fertilization with male gamete?	a. Uterus b. Fallopian tube c. Ovary d. All the above
Q4. What is the normal length of one menstrual cycle (in days)?	a. 15-25 b. 21-35 c. 25-40 d. 30-45
Mark symptoms seen in PCOS as true or false	Q5. Delayed menstruation Q6. Acne Q7. Dark, velvety & thickened skin folds Q8. Weight loss Q9. Inability to conceive
Q10. Which organ regulates the hormonal balance for growth and menstruation in female?	a. Pituitary glands b. Pancreas c. Kidney d. Liver
Q11. What is the recommended daily calorie intake to reduce weight in patient with PCOS with an overall sedentary lifestyle?	5000 calorie/day 2100 calorie/day 1200 calorie/day 3300 calorie/day
Q12. Food to be avoided by patients with PCOS	French fries Red meat Processed food Refined sugars All of the above
Q13. How do alternative medicine practices like Yoga and meditation help in women with PCOS?	a. Increasing weight and fertility, b. Reducing weight and stress, c. Reducing weight & stress and increasing fertility, d. None of the above.
Q14. Which of the following symptoms may need medical treatment, if not checked by lifestyle modification ?	a. Delayed menstruation b. Painful menstruation c. Weight loss d. All of the above

Table 2. Continued

Mark following statements as correct/incorrect regarding PCOS	Q15. Obesity is modifiable cause of PCOS, Q16. Lifestyle modifications (Weight control and regular exercise) and pharmacotherapy can prevent complications of PCOS, Q17. Women with PCOD have high risk of diabetes in later life, Q18. Nutritional management has no role in treatment of PCOS, Q19. Polycystic ovary and ovarian cancer are same, Q20. Anovulation is not the cause of infertility in PCOS women.	
Q21. Women with PCOS are at risk of	a. Breast cancer b. Stomach cancer c. Uterine cancer d. Blood cancer	
Match the following drugs in management of PCOS	Q22. Metformin Q23. Anti-androgenic drugs Q24. Hormonal pills Q25. Ovulation induction drugs	a. Insulin Insensitivity b. Infertility c. Acne d. Irregular menstruation
PCOS: Polycystic ovary syndrome		

Scoring

Responses from the participants were scored using a model answer key. A score of "one" or "zero" was awarded for each correct or incorrect answer, respectively, and the sum total for each participant was expressed as the "awareness score" described on an ordinal scale as follows: fair (10 or less), moderate (11-17) and good (18-25).

The maximum achievable score was 25. The difference in awareness scores between the pre-exposure and post-exposure responses was quantified to investigate the effectiveness of the learning modules in increasing knowledge.

The questions were categorised into the following domains based on content. Domain 1 (10 questions) included questions related to the normal menstrual cycle and pathophysiology of PCOS. Domain 2 focussed on the importance of lifestyle management (7 questions) and Domain 3 (8 questions) dealt with the indications for pharmacological intervention and role of treatment adherence in PCOS.

Statistical analysis

Descriptive statistics was used for qualitative data and paired t-test and z-test were used, as appropriate, to compare outcomes. Effect size was estimated to assess magnitude of effect on knowledge due to intervention (small, medium, or large) (10). Statistical analysis was done manually using MS Excel 2016.

Results

A total of 41 eligible participants completed both pre- and post-test questionnaires. Most women (92.6%) belonged to middle or lower socio-economic classes and all had completed a minimum of higher secondary education. Age distribution of the participants was 20% aged 18-21 years, 39% aged 22-26 years, and 41% aged 27-35 years. Only 29.2% of the women primarily attended clinic for infertility while the rest sought consultation for menstrual irregularities.

Mean ± standard deviation awareness score prior to intervention was 15.09±4.31, which increased to 18.60±3.85 after the intervention. This increase indicated a better understanding of the disease condition when tested using the paired t-test ($t=9.6722$; $p=0.00001$). Younger participants, aged ≤26 years scored better with higher pre- and post-test scores (Table 3). Most (48.8%) of the respondents had baseline awareness in the "moderate" range (scores between; 11-17) whereas post intervention scores improved to the "good" category for 63.4% of the women (Table 4, 5).

Table 3. Awareness scores

	Age (years)	n	Pretest score (mean ± SD)	Post test score (mean ± SD)	Paired t-test
All respondents	18-35	41	15.09±4.31	18.60±3.85	t=9.6722; p=0.00001
Age subset	18-21 22-26 27-35	16 18 7	16.44±2.96 16±3.92 9.71±4.19	19.50±3.05 18.83±4.25 16±3.78	t=9.1411; p=0.00001 t=14.0185; p=0.00001 t=3.8590; p=0.0083
SD: Standard deviation					

Table 4. Awareness score

	Number of women in each category based on awareness score (n)	
	Pre-test	**Post-test**
Fair (10 or less)	7 (17.07%)	3 (7.32%)
Moderate (11-17)	**20 (48.79%)**	12 (29.27%)
Good (18-25)	14 (34.14%)	**26 (63.41%)**

Table 5. Question domain-wise responses in pretest and post-test

Question groups	% Respondents who answered correctly (pre-test)	% Respondents who answered correctly (post-test)	Z-test
Domain 1	75	91	p<0.00001
Domain 2	57	86	p<0.00001
Domain 3	52	70	p<0.00001

Furthermore, effect size was calculated for the pre- and post-intervention data sets using Cohen's d value. Cohen's d was 0.85, meaning intervention with teaching modules had a significantly large effect on knowledge, suggesting effectiveness of the teaching method.

Feedback received from the participants was positive. The majority (90.2%) were satisfied with the consultation experience and 92.7% agreed that the video helped them gain new perspectives towards their disease.

Discussion

The study was done to assess the educational value of a structured teaching method in raising awareness of PCOS in an outpatient setting. The educational module was effective in increasing awareness and changing subjective perspective about PCOS as demonstrated by a significant increase in the overall awareness score. In addition, the intervention was shown to have a significant impact of patients' understanding of their condition, as demonstarted by the Cohen's d value. Results of the present study are consistent with previous studies where a similar structured teaching program has shown been shown to have a significant impact on disease knowledge in participants from varied educational backgrounds (11-14).

Participation in the present study required a minimum level of literacy, economic stability (possession of a smart phone or computer) and familiarity with the internet. These socio-economic factors may be reflected in the relatively higher levels of baseline awareness demonstrated by the participants with an average pre-test score of 15.09. Higher pretest scores noted in the two younger age groups suggest changing age-related societal level processes, in part due to an increase in the use of the internet for information and communication amongst young people. However, the quality of information available from the internet and other commercial entities is known to be inconsistent and lacking credibility (15,16).

Empowering women by increasing health literacy becomes even more crucial for those from disadvantaged backgrounds with little or no access to reliable information. Structured teaching remains relevant in such populations, which includes adolescent girls from rural India. A meaningful gain in knowledge has been reported using teaching modules customized to accommodate local culture and perceptions (13,14).

PCOS is a chronic multisystem disorder with considerable variation in symptom expression. Lifestyle change and nutritional management remain the first line of management for all, even in women with a lean PCOS phenotype, as there is a strong association between abdominal obesity and insulin resistance in women with PCOS who are not markedly overweight (17). Lifestyle change is multifactorial and includes goal setting, self-monitoring, stimulus control, slower eating, reinforcing changes, and prevention of relapse to optimise physical and emotional health in women (18). Even modest reform of an individual's approach to nutrition and exercise drastically improves endocrine features, reproductive function and cardiometabolic risk profile, even without marked weight loss (19). A key shift in cognitive behaviour should be the goal, as short-term diets, exercise and therapies rarely lead to a permanent effect. The message should be emphasized at every clinic visit and customized teaching tools should be used routinely to reinforce it.

The present study demonstrated the positive impact of a suitable and well-timed intervention, in this case patient education occurred at the time of consultation, in increasing patient awareness, which can translate into long term behaviour change. Though direct and indirect evidence about this is available, focus on disease literacy during consultation is not routine and practical information related to lifestyle for symptom management and preventing long-term complications of PCOS, is not often provided (8,15,16,20,21). Introduction of a brief but focussed educational element in outpatient settings involves almost no cost after development and little inconvenience. Where appropriate, the managing clinician should take on the primary responsibility for educating patients, to ensure continued understanding of the disorder, life course implications, engagement in lifestyle improvement, and participation in regular screening for metabolic complications (21). Consultation visits may be the best time to educate and reinforce behavioural change, as patients are more receptive to the inputs with respect to

functional understanding of the diagnosis, role of continued care and long term implications. This study provided evidence of a significant change in awareness and perception of PCOS that was achieved from a small intervention with minimal effort. This may be important in improving long term health outcomes in PCOS.

Study limitation

The main limitation of the study was the small and homogenous sample, as recruitment was from a single centre. However, the large effect size, even in such a small homogeneous group, suggested the possible utility of this approach in a more heterogeneous group of participants. To test this hypothesis there would be a need for a set of validated teaching modules adapted to local language, customs, and cultural perceptions, that would be accessible by a wider population. A subsequent comparative analysis on heterogeneous groups of participants would be needed. Inclusion of a control group with crossover design would further increase internal validity.

Conclusion

Patient education using simple teaching tools during routine consultation provided an opportunity to improve patients' knowledge of PCOS and the life course implications for PCOS. Empowering patients by improving disease literacy will promote preventive aspect of health care. This is important in the management of this chronic disorder, PCOS.

Ethics Committee Approval: *A longitudinal study using one group pretest-posttest design was conducted as a student project, funded by the Indian Council for Medical Research after approval from the All India Institute of Medical Sciences Bhopal Madhya Pradesh India, Institutional Human Ethics Committee and Review Board (approval number: IHEC-LOP/2018/STS0151).*

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

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Comparison of maternal serum NRG-4 levels in healthy and preeclamptic pregnancies

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Abstract

Objective: The new adipokine, neuregulin-4 (NRG-4), acts as a signaling protein and plays a role in lipogenesis, inflammatory events and atherosclerosis. The aim was to investigate maternal levels of NRG-4 in preeclampsia (PE) disease.

Material and Methods: Pregnant women with PE, divided into severe and mild PE, and gestational age-matched healthy pregnant women, as a control group, were recruited. NRG-4 levels were measured using an ELISA. NRG-4 levels in the groups and the relation between NRG-4 and clinical and laboratory parameters were analyzed.

Results: There were 41 women in the PE group, 11 (26.8%) in the severe and 30 (73.2%) in the mild subgroups and 41 controls. There were no significant differences between the groups in terms of maternal age, gravidity, parity, abortion, gestational week at the time of blood sampling, levels of hemoglobin, platelet count, alanine and aspartate transaminases ($p=0.067$, $p=0.819$, $p=0.957$, $p=0.503$, $p=0.054$, $p=0.217$, $p=0.306$, and $p=0.270$ respectively). The PE group had higher body mass index, nitrogen urea and creatinine values, and diastolic and systolic blood pressure ($p=0.005$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$ respectively). In addition, earlier gestational week at delivery, lower birth weight and Apgar scores at 1 and 5 minutes and the occurrence of non-reassuring fetal heart rate tracing were found in the PE group ($p=0.010$, $p=0.004$, $p=0.005$, $p=0.005$, and $p=0.026$ respectively). There were no significant differences between the groups in terms of NRG-4 ($p=0.611$). No correlation was identified between clinical parameters examined and NRG-4 levels ($p=0.722$).

Conclusion: No association was found between NRG-4 concentrations and PE patients, regardless of severity of PE, compared to healthy pregnancies. Future longitudinal studies are needed to confirm this lack of association in PE. (J Turk Ger Gynecol Assoc 2022; 23: 8-13)

Keywords: NRG-4, neuregulin, preeclampsia, perinatal outcome

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Introduction

Preeclampsia (PE) is a serious cardiovascular disorder of pregnancy, which is characterized by hypertension in addition to proteinuria or hypertension and end-organ dysfunction in the absence of proteinuria (1). The course of the disorder is unpredictable, and associated with neonatal and maternal life-threatening complications (1-3). The pathogenesis of PE is multifactorial and involves abnormal development of placenta, endothelial dysfunction, and immunological and genetic factors (4-6). Changes in the balance of angiogenic and antiangiogenic factors, and the presence of insulin resistance and/or obesity contribute to the pathogenesis of the PE and clinical symptoms (7).

Neuregulins are members of the endothelial growth factor-like growth factor family. Four subtypes of neureglins have been identified, one of which is neuregulin-4 (NRG-4) (8). NRG-4 is mainly produced by brown adipose tissue and plays a role as signaling protein during cell-to-cell interaction (9). In vivo studies have suggested that NRG-4 levels change during the process of lipogenesis, inflammatory events and as a result of changes in energy metabolism (10,11). It has been suggested that NRG-4 positively correlates with the development of obesity related disorders, such as type 2

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diabetes mellitus (DM) and non-alcoholic fatty liver disease (NAFLD) and plays a role in the development of coronary artery disease (CAD) by promoting atherosclerosis (12-17).

The relationship between adipokines and metabolic disorders is complex and is not fully understood. In addition to adipose tissue, placental tissue is thought to be a source of adipokines. The relationship between metabolically active proteins, such as leptin, resistin, adiponectin and tumor necrosis factor-α has been investigated in the pathogenesis of PE. However, no clear relationship between adipokine levels and PE has been found (18-21). Therefore, the aim of this study was to investigate if there was an association between PE and NRG-4 levels for the first time. We also aimed to understand whether NRG-4 levels are associated with the severity of PE and neonatal and maternal clinical parameters.

Material and Methods

Study participants

This was a case-control study. Pregnant women with PE, divided into two subgroups as severe PE and mild PE, and gestational age-matched healthy pregnant women as a control group were recruited. All data were collected between September 2018 and March 2019 at the Department of Perinatology of Zekai Tahir Burak Women's Health Training and Research Hospital in Ankara, Turkey. The study design was approved by the institutional research ethics committee (approval number: 28/2019) and written informed consent was obtained from all participants. The study was performed according to the universal principles expressed in the Declaration of Helsinki. All participants were in the third trimester of pregnancy. Exclusion criteria included any patient having: a chronic systemic disease; an autoimmune disease; chronic drug use; or presence of multiple gestation; presence of fetal congenital abnormality; and presence of complication of pregnancy including gestational DM, chorioamnionitis, and premature preterm rupture of pregnancy. Body mass index (BMI) was calculated as body weight (in kilograms) divided by squared height (in metres). According to the ACOG guideline (1), the criteria for the diagnosis of PE and in which cases it is called PE with severe features (the group called severe PE according to the old nomenclature) are given below.

Diagnostic criteria for preeclampsia:

- New onset of hypertension, defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg in at least two measurements over four hours or six hours apart, or systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg confirmed within a short interval (minutes).

and

- Proteinuria defined as protein/creatinine ratio ≥0.3 mg/dL, dipstick reading of 2+ or ≥300 mg in a 24-hour urine collection after 20 weeks of gestation,

or

- In the absence of proteinuria - new onset of hypertension with signs of end-organ dysfunction, such as platelet count <100,000/μL, increased liver transaminases on at least two occasions, pulmonary edema, serum creatinine >1.1 mg/dL or two-fold elevation of basal creatinine level or new-onset headache, unresponsive to medication, or visual symptoms

The presence of any of the following criteria was grouped as "severe PE" (according to the old nomenclature, and "severe PE with severe feature" according to the new nomenclature).

PE with severe features as following:

- Systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on two occasions at least 4 hours apart (unless antihypertensive therapy was initiated),
- Platelet count <100,000/μL,
- At least two episodes of increased liver transaminases,
- Pulmonary edema,
- Serum creatinine >1.1 mg/dL or there was a two-fold elevation of basal creatinine level,
- New-onset headache unresponsive to medication,
- Visual symptoms,
- Severe persistent right upper quadrant or epigastric pain unresponsive to medications (Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222 Obstet Gynecol 2020; 135: e237-60. doi: 10.1097/AOG.0000000000003891).

Based on the ACOG guideline (1), patients who met the above-mentioned PE diagnostic criteria but did not have the criteria of PE with severe features; were classified as mild PE group (mild PE with the old nomenclature, PE without severe features with the new nomenclature).

Blood samples

All blood samples were taken from the antecubital vein. Samples for the measurement of NRG-4 levels were taken into tubes containing ethylene diamine tetra-acetic acid blood samples of 5 mL volume were centrifuged for 10 minutes at 1,000×g at 2-8 °C within 30 minutes of collection. Then plasma was stored at -80 °C until analysis. Concentrations of alanine transaminases, aspartate transaminases, creatinine, urea, hemoglobin and platelet count were measured as routine laboratory parameters. NRG-4 levels were measured using an ELISA (Human NRG-4 ELISA Kit, Cloud-Clone Corp., Katy, TX 77494, USA) following manufacturer's instructions. The intra- and inter-assay coefficients of variation were <10% and <12%, respectively. The detection range was 0.156-10 ng/mL. The

minimum detectable dose of NRG-4 is typically less than 0.056 ng/mL.

Statistical analysis

IBM SPSS Statistics, version 21.0 (IBM Corp. Armonk, NY, USA) was used to analyze the collected data. Kolmogorov-Smirnov test was used to evaluate the closeness of data sets to normal distribution. Descriptive statistics were expressed as mean ± standard deviation and median (minimum-maximum). The parametric Sample t-test and non-parametric Mann-Whitney U test were used to determine statistically significance between two independent groups, as appropriate. Comparison of two qualitative variables was done with the chi-square test, according to the expected value levels. Spearman and Pearson correlation tests were used to examine the association between two variables. Statistical significance was assumed when $p<0.05$.

Results

Eighty-two women participated in this case-control study, equally divided between the PE group (n=41) and control group (n=41). Clinical and demographic parameters of study groups are shown in Table 1. There were no significant differences between the PE and control groups in terms of maternal age, gravidity, parity, abortion and gestational week at the time of blood sampling. BMI was significantly higher in the PE group compared to the control group. Similarly, there was no difference between the PE and control groups in terms of hemoglobin concentration, platelet count, and alanine and aspartate transaminases levels. However, renal function tests such as blood nitrogen urea and creatinine were significantly elevated in the PE group in comparison with the control group (Table 1).

Perinatal outcomes of the study groups are shown in Table 2. Gestational week at the time of delivery was earlier in the PE group than in the control group. Babies born to mothers in the control group had significantly elevated birth weight compared to babies born to mothers with PE. Mothers with PE were more likely to deliver by cesarean section when compared with controls. Apgar scores at 1 minute and 5 minutes were much lower in babies from the PE group but there was no difference between the groups in terms of neonatal intensive care unit admission likelihood (Table 2).

The PE group was divided into severe (n=11, 26.8%) and mild (n=30, 73.2%). Statistically, there was no difference in terms of NRG-4 levels between the PE group as a whole and the control group (Table 1). In addition, there was no difference in NRG-4 levels between the severe and mild PE groups (p=0.72). As shown Table 3, no significant correlations were identified between NRG-4 levels and clinical, laboratory and demographic parameters.

Discussion

NRG-4, a new brown adipose tissue-associated adipokine, has been reported to play an important role in the regulation of energy metabolism and in the development of obesity related diseases (12-16). Besides acting in paracrine and autocrine

Table 1. Demographic and laboratory parameters of study groups

	Preeclampsia group (n=41)	Control group (n=41)	p
Maternal age, years	30.0 (18.0-43.0)	29.0 (19.0-39.0)	0.067
Gravidity (number)	2.0 (1.0-6.0)	2.0 (1.0-6.0)	0.82
Parity (number)	1.0 (0.0-3.0)	1.0 (0.0-3.0)	0.96
Abortion (number)	0.0 (0.0-3.0)	0.0 (0.0-3.0)	0.50
BMI (kg/m^2)	31.27±6.55	27.90±3.68	**0.005**
Gestational age at the time blood sampling, weeks	35.0 (25.0-41.0)	36.0 (26.0-41.0)	0.054
SBP (mmHg)	149 (140-189)	109 (85-128)	**<0.001**
DBP (mmHg)	89.83±11.0	61.93±7.79	**<0.001**
Hemoglobin (g)/dL	12.05±1.57	11.70±1.03	0.217
AST (U/L)	16.0 (8.0-62.0)	14.5 (8.0-26.0)	0.306
ALT (U/L)	10.0 (4.0-80.0)	9.0 (6.0-20.0)	0.270
BUN (mg/dL)	21.5 (8.0-50.0)	13.5 (7.0-33.0)	**<0.001**
Creatinine (mg/dL)	0.6 (0.1-1.0)	0.5 (0.4-0.8)	**<0.001**
Platelet (10^3/mL)	232 (30.0-363)	221 (116-540)	0.899
Serum NRG-4 level (ng/mL)	1.5 (1.0-6.6)	1.6 (0.1-3.5)	0.611

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, NRG-4: Neuregulin-4

Table 2. Perinatal outcomes of preeclampsia and control groups

	Preeclampsia group (n=41)	Control group (n=41)	p
Gestational age at delivery (week)	36 (28-40)	38 (29-41)	**0.010**
Birth weight (gr)	2355 (450-3920)	3000 (1220-3930)	**0.004**
Apgar score at 1 minute	7 (6-8)	8 (7-9)	**0.005**
Apgar score at 5 minutes	9 (7-10)	10 (9-10)	**0.005**
C/S rate	34/41 (82.9%)	9/41 (21.9%)	**<0.001**
Non-reassuring fetal heart rate trace	10/41 (24.3%)	2/41 (4.8%)	**0.026**
NICU admission	12/41 (29.3%)	8/41 (19%)	0.411
NICU: Neonatal intensive care unit, C/S: Cesarean section			

signal transduction, NRG-4 decreases hepatic lipogenesis and increases fatty acid beta-oxidation in an endocrine fashion (9). Thus, NRG-4 contributes to lipid and glucose homeostasis. It has been reported that NRG-4 promotes the development of obesity-related disorders, such as type-2 DM and NAFLD (12-16). In addition to the metabolic roles ascribed to NRG-4, Ma et al. (21) suggested that excessive production of NRG-4 may have anti-atherogenic and anti-inflammatory effects. Similarly, it has been suggested that decreased NRG-4 levels may induce the development of atherosclerosis (20). Sato and Minatsuki (17) reported that NRG-4 levels were lower in patients with CAD than in the control group. However, it has not been clearly established which metabolic pathways NRG-4 interacts with. Activating ERB B4, a member of the epidermal growth factor receptor and plays role in the diseases such as cancers, DM may lead to the progression of atherosclerosis by inhibiting the apoptosis of endothelial cells via NRG-4 (14). It has also been shown that reduced NRG-4 levels were related to increased carotid intima thickness and carotid plaque in a group with obesity when compared to a control group (22). In the light of this evidence, we investigated whether there was an alteration of NRG-4 levels in women with PE. This seemed a reasonable hypothesis, given that PE is known to be associated with obesity, endothelial dysfunction, inflammation and metabolic diseases. Considering the similarities between mechanisms and risk factors, it was expected that there would be a correlation between NRG-4 and PE. However, we did not find any such relationship. As has been previously reported, we found higher BMI in the PE group, but there were no difference in NRG-4 levels between the groups. Furthermore, there was no correlation between NRG-4 level and the severity of PE, nor with clinical and laboratory parameters. It is thought that NRG-4 is a marker of brown adipocytes in human adipose tissue and is also associated with obesity. No such relationship was evident in after analysis of the data from our study populations; we could not find any correlation between NRG-4 levels and BMI.

Table 3. The correlation between NRG-4 and clinical, laboratory and demographic parameters in the participants

	NRG-4 concentration	
Variables	**r***	**p**
Maternal age, years	-0.165	0.136
Gravidity (number)	-0.021	0.848
Parity (number)	-0.019	0.862
Abortion (number)	0.083	0.454
BMI (kg/m^2)	-0.166	0.134
SBP (mmHg)	0.001	1.000
DBP (mmHg)	-0.141	0.380
Hemoglobin (g)/dL	0.004	0.975
AST (U/L)	0.131	0.239
ALT (U/L)	0.093	0.407
BUN (mg/dL)	-0.080	0.477
Creatinine (mg/dL)	-0.066	0.554
Platelet (10*3/microL)	-0.056	0.618
Birth weight (gr)	-0.061	0.582
Gestational age at delivery (week)	-0.082	0.461
Birth weight (gr)	-0.048	0.669
Apgar score at 1 minute	0.010	0.928
Apgar score at 5 minutes	0.023	0.837
BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, NRG-4: Neuregulin-4		

There may be a number of possible reasons why these findings emerged from our study. Firstly, the pathogenesis of PE involves a dynamic process, so there may be temporal changes in concentrations of circulating cytokines and adipokines as the pathogenic process progresses. These cytokine/adipokine concentrations may be normalized again by the time clinical symptoms and overt PE appears. However, it is known that obesity, especially with intensive visceral adiposity, can contribute to the pathogenesis of PE by increasing proinflammatory cytokines and adipokines. There have been contrasting reports of the utility of assessing concentrations of

visceral mass-derived adipokines (such as resistin, visfatin) or adipokines reflecting general adiposity (adiponectin, leptin) for the prediction of the development of PE (18,20,23-25). In the study of Chandrasekaran et al. (19), it was demonstrated that PE was associated with elevated level of visceral fat mass-derived adipokines and leptin, but there was no relation between the groups for adiponectin levels. These analyses bring into question the importance of the effect of placentally derived adipokines in the pathogenesis of PE.

Secondly, although higher BMI is known to contribute to developing PE and there is correlation between BMI and increasing PE severity. BMI, which is especially affected by white adipose tissue, may not fully reflect the increased brown adipose tissue. Therefore, BMI may be an insufficient indicator to reflect body fat tissue distribution. Chandrasekaran et al. (19) showed that normal weight and obese women in the PE and control groups had similar levels of visceral mass-derived adipokines, cytokines and inflammatory markers. Kurek Eken et al. (26) demonstrated that serum NRG-4 levels were higher in patients diagnosed with gestational DM compared to healthy pregnant women. Moreover, they reported that NRG-4 concentration was positively correlated with BMI, and triglyceride and low-density lipoprotein cholesterol. Jiang et al. (22) suggested that low NRG-4 levels were associated with increased subclinical atherosclerosis and increased carotid intima thickness in obese patients. They also showed that among the obese patients, those with high NRG-4 levels had lower BMI and systolic blood pressure levels than those with low NRG-4 levels. In the study conducted by Dai at al. (16) decreased NRG-4 levels were found in NAFLD and yet they did not find any relationship between NRG-4 and BMI. Similarly, Sato and Minatsuki (17) showed that NRG-4 is a predictor of the severity of CAD but they did not find any correlation between NRG-4 and BMI, cholesterol levels or high sensitive C-reactive protein. Similar to these studies, we did not detect any correlation between the demographic, clinical and laboratory parameters and NRG-4 levels and the severity of PE was also not related with NRG-4 levels. Therefore, it may be assumed that NRG-4 levels are independent of general measures of obesity or that BMI does not accurately reflect the presence and activity of brown adipose tissue, the main source of NRG-4. It is also possible that NRG-4 levels may be increased in PE pregnancies earlier than the third trimester, when samples were taken in our study.

It should be noted that brown adipose tissue plays a role in energy metabolism by regulating the production of ATP and thermogenesis (10,11). Similarly, Wang et al. (10) demonstrated that NRG-4 stimulates liver lipogenesis by activating Erb B3/B4 receptors. This evidence suggests that the endocrine role of NRG-4 in metabolic diseases, such as type 2 DM, gestational DM, NAFLD and obesity may be more closely associated with vascular and inflammatory pathways. All of these factors, particularly the low levels of NRG-4 associated with an atherogenic process, which has similarity with the etiopathogenesis of PE and, conversely, elevated levels in gestational diabetes mellitus, which also has some similarities to PE, may be the reason why we could not detect any significant variation in NRG-4 levels among the study and control groups. We therefore suggest that NRG-4 is an unsuitable biomarker for third trimester PE.

Study limitations

However, there were limitations of this study that should be noted. One problem lies with sampling time and the lack of data for NRG-4 levels prior to the onset of clinical PE. Samples in our study were taken only in the third trimester and longitudinal sampling throughout preganacy could have provided more enlightening results. These data collected in a longitudinal fashion may provide a greater understanding of the significance of NRG-4 during the development of PE. A further limitation was the relatively small sizes of the PE sub-groups. Future studies should recruit sufficient patients with PE to sub-divide them on the basis of both obesity and severity of disease. These data may add more detailed and reliable information about the pathogenesis of PE and the role of NRG-4, if any, in this.

Conclusion

In conclusion, despite the limitations, to our knowledge, this is the first study to have investigated maternal levels of NRG-4 in PE. Though we could not find any difference in NRG-4 levels in PE pregnancies compared to healthy pregnancies, future investigations of the role of NRG-4 in PE should address the physiological changes of pregnancy, metabolic pathways known to be affected by NRG-4 and the different stages in the development of PE.

Ethics Committee Approval: *The study design was approved by the institutional research ethics committee (approval number: 28/2019).*

Informed Consent: *Written informed consent was obtained from all participants.*

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The effects of intra-ovarian autologous platelet rich plasma injection on IVF outcomes of poor responder women and women with premature ovarian insufficiency

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Abstract

Objective: There are controversial results regarding the administrations of platelet rich plasma (PRP) to increase in-vitro fertilization (IVF) success rates in the current literature. The aim of this study was to evaluate the effects of intra-ovarian PRP injections on IVF outcomes of poor responder women and women with premature ovarian insufficiency (POI).

Material and Methods: The medical history and outcome of women receiving intra-ovarian PRP injections performed in a single tertiary center between 2018 and 2021 was retrospectively reviewed.

Results: In total 71 women were included, of whom 21 were diagnosed with POI according to European Society of Human Reproduction and Embryology criteria and 50 were poor responders according to Bologna criteria. Number of retrieved oocytes, number of 2 pronuclear embryos and number of cleavage stage embryos were significantly higher in poor responder women after PRP injections. However clinical pregnancy rates and live birth delivery rates were similar before and after PRP injections in poor responders. In women with POI, 8 embryos were obtained in cycles commenced after PRP injections but no clinical pregnancies were achieved in this group of patients.

Conclusion: Intra-ovarian PRP injections do not appear to increase live birth rates or clinical pregnancy rates in poor responder women or in those with POI, in this cohort. (J Turk Ger Gynecol Assoc 2022; 23: 14-21)

Keywords: Platelet rich plasma, poor responder, in-vitro fertilization, premature ovarian insufficiency

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Introduction

Decreased ovarian reserve and premature ovarian insufficieny (POI) are two entities that dramatically lower the chances of conception with assisted reproductive technologies. The problem stems from the low or absent oocyte yield, that usually cannot be improved by any current techniques.

POI is defined as loss of ovarian functions before the age of 40 years by the European Society of Human Reproduction and Embryology (ESHRE) (1). POI is estimated to have a prevalence of about 1% in the general population and it is a challenging condition for both patients and the physicians (1). Although pregnancies may occur in 5-10% of women with POI, either spontaneously or by in-vitro fertilization (IVF), oocyte donation remains the only treatment option for most patients (2). A range of treatment modalities are suggested to improve ovarian function and to achieve pregnancies without using donor eggs in these patients, including stem cell therapies and ovarian tissue auto-transplantation, although the outcomes have been unsatisfactory (3-6).

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For decades, the definition of poor ovarian response was not standardized and studies had been conducted using different criteria. There is now an accepted definition. Low ovarian response is currently defined as ≤3 ovarian follicles on the day of oocyte maturation triggering or ≤3 oocytes obtained in a controlled ovarian stimulation cycle (7). Low ovarian response constitutes 9% to 18% of IVF/embryo transfer cycles (8). These patients have poorer prognosis with live birth rates ranging from 6% to 23% in different studies (9,10). Some of the attempts to improve the oocyte yield by changing the ovarian stimulation protocol, gonadotropin dosage, gonadotropin type, pretreatment use of androgens, and so forth failed to result in better outcomes. Unsuccessful IVF attempts caused by low ovarian response brings additional frustration on already distressed couples.

More recently, innovative approaches, such as in vitro oocyte activation (IVA) which involves harvesting ovarian tissue and treating it with phosphatase and tensin homolog (PTEN) inhibitors in vitro, also seems not to be very efficient, although there have been some miracelous outcomes (6,11). As less labor-intensive approaches, some other treatment alternatives have emerged with yet unproven efficiency. These include ovarian injection of autologous platelet rich plasma (PRP).

PRP is a blood product containing high concentrations of platelets, a range of cytokines and growth factors, such as platelet derived growth factor, vascular endothelial growth factor (VEG-F), epidermal growth factor, transforming growth factor-beta (TGF-β) and insulin like growth factor-1 and 2 (IGF-1, 2). Source of cytokines in PRP solution could either be platelet degranulations as well as mechanical lysis of other blood cells. PRP is shown to induce angiogenesis, tissue regeneration, activate anabolic pathways for cell proliferation and differentiation, and aids in homing of stem cells (12). This new modality is increasingly used for regenerative purposes in dermatology, orthopedics and aesthetic surgery (13). Owing to the proposed mechanism of action, ovarian injection of PRP is hypothesized to promote ovarian rejuvenation. The rationale for this procedure is based on concentrating the soup of cytokines and growth factors associated with PRP and directly injecting them into ovarian tissue in an attempt to improve ovarian function. Some studies have reported increased ovarian angiogenesis, folliculogenesis, restored menstrual cycles and improved ovarian function tests following ovarian PRP injections (14,15). Although these findings drew attention to ovarian PRP injections in the treatment of infertile patients with poor prognosis, data about the effectiveness of this new modality is scarce, particularly in terms of the ultimate goal of assisted reproduction: live birth delivery rates.

In this study, the outcomes and efficacy of ovarian PRP injections performed for IVF purposes were evaluated retrospectively.

Material and Methods

Patients who underwent ovarian PRP injection due to POI or poor ovarian response in previous cycles in a university affiliated infertility center between 2018 and 2021 were retrospectively evaluated. Data was obtained from hospital records. Ethical approval for this study was obtained from Üsküdar University Faculty of Medicine at 28/05/2021 (approval number: 61351342/MAY 2021-04). The study protocol conformed to the "Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects" and the need for consent was waived by the ethical committee due to the retrospective design.

ESHRE criteria used for the diagnosis of POI are at least four months of amenorrhea and elevated follicle-stimulating hormone (FSH) >25 U/L in patients younger than 40 years of age. The Bologna criteria were adopted for the study definition of poor responders. To be defined as a poor responder by the Bologna criteria, at least two of the following three criteria should be met: 1) age >40 years; 2) poor ovarian response in previous IVF cycles (≤3 oocytes retrieved in a conventional stimulation protocol); and 3) abnormal ovarian reserve tests.

In our institution, documented fixed standards are used to prepare and apply PRP. A total of 20 mL of blood is collected from each patient into two tubes. T-LAB PRP kit (T-Biotechnology, Bursa, Turkey) is used to prepare the PRP. Tubes are centrifuged at 1500 g for eight minutes. Approximately 2 mL of plasma is gathered above the newly formed buffy coat layer from each tube through a 16 G needle into a 5 mL syringe. Plasma obtained from the tubes is transferred into a single re-suspension tube and gently agitated for 30-60 seconds to prepare the PRP solution for use. A total of 4 mL of PRP solution was obtained per patient and divided into two equal portions to inject into each ovary. Patients were sedated for ovarian injection. The procedure was carried on with a 35 cm long 17 G needle under transvaginal ultrasound guidance. 2 mL of solution was injected into the stromal region of each ovary within two hours of PRP preparation.

Women were assessed monthly for menstrual status, antral follicle count and serum hormone levels for at least six months following PRP. Monitoring started at the first mensturation following PRP injection. Controlled ovarian stimulation was initiated in patients that were found eligible within the first five days of the menstrual cycle. Recombinant (rFSH, Gonal-F, Merck Serono S.p.A), human menopausal gonadotropin (hMG, Merional, IBSA Institut Biochimique S.A, Menopur® Ferring Pharmaceuticals) or a combination of recombinant luteinizing hormone and rFSH (Pergoveris, Merck Serono SA) was used for ovarian stimulation, as per practitioner's choice. Patients were monitored during stimulation for follicular growth with serial transvaginal ultrasounds and serum

hormone levels. Adjustments in gonadotropin doses were made in accordance with each patient's follicular growth. Once the leading follicle reached a diameter of 12-14 mm, gondatropin releasing hormone (GnRH) antagonist (Cetrotide 0.25 mg, Pierre Fabre Medicament Production) injections were commenced to suppress premature LH peak and continued to the day of oocyte maturation triggering. A dual-trigger method was used to induce oocyte maturation with a GnRH agonist of 0.2 mg triptorelin acetate, (Gonapeptyl, Ferring Pharmaceuticals) and 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) when at least one follicle had reached a diameter of 18 mm. Oocytes were retrieved under transvaginal ultrasound guidance 35-36 hours after oocyte maturation trigger. Fertilization was conducted by intracytoplasmic sperm injection. Developing embryos were graded according to İstanbul consensus workshop guidelines (16). Day 3 or day 5 embryos were transferred using an embryo transfer catheter under abdominal ultrasound guidance. A maximum of two embryos were transferred in each attempt. Luteal phase support was initiated in every patient with 200 mg intravaginal progesterone (Lutinus, Ferring Pharmaceuticals) twice a day and continued through the eight to tenth gestational weeks.

Exclusion criteria included: patients with high (>30 kg/m^2) or low (<18 kg/m^2) body mass indices (BMI); patients with additional endocrine disorders (thyroid dysfunction, hyperprolactinemia, diabetes mellitus, Addison disease, congenital adrenal hyperplasia, Cushing syndrome); patients with corrected or present uterine anomalies; and patients with infertility due to azoospermia. Seventy-one women were recruited for ovarian PRP injection within the selected period of time for POI and poor ovarian response. Twenty-one of them were defined as POI, and two were lost to follow-up and excluded. Fifty women were defined as poor responders by the Bologna criteria. All of the poor responders had a history of previous ovarian stimulation cycle that resulted in ≤3 oocytes being retrieved. Outcomes of IVF cycles before and after PRP administration were compared in poor responders and cycle outcomes following ovarian PRP injection in women with POI were assessed. Our primary outcome was live birth delivery rates. Live birth was defined as live infants delivered after the 24th gestational week. Secondary outcomes were: number of oocytes retrieved; number of metaphase 2 (M2) oocytes; fertilization rates [2 pronuclear embryos (2PN)/M2 oocytes]; number of cleavage stage embryos; and implantation rates (gestational sacs observed/transferred embryos). Outcome parameters were defined in accordance with The International Glossary on Infertility and Fertility Care, 2017 (17).

Statistical analysis

Statistical analysis was done using IBM SPSS, version 23 (Evaluation version; IBM, Armonk, NY, USA). Descriptive statistics are expressed as mean ± standard deviations for normally distributed data and as median (minimum-maximum) for non-normally distributed data. Categorical variables are expressed as numbers and percentages (%). Significance of differences in means and medians among groups were assessed by Student's t-test and Mann-Whitney U test, respectively. Categorical variables were evaluated with Pearson's chi-squared test or Fisher's exact test. A p-value <0.05 was considered significant.

Results

A total of 71 women who underwent ovarian PRP injection within specified period of time were eligible for the study. PRP injection was performed in 50 women because of poor ovarian response in previous IVF cycles and to 21 women due to POI. Two women with diagnosis of POI lost follow-ups and excluded from the study. Mean age and BMI of patients with POI were 37.9±1.9 years and 24.9±3.1 kg/m^2, respectively. In poor responders mean age was 38.1±4.4 years and mean BMI was 25±3.4 kg/m^2.

In 10 (52.6%) of 19 POI cases, menstruation was restored following PRP and controlled ovarian stimulation cycles could be commenced. Mean interval betweeen PRP injections and the start of menstral cycles was 3.1±0.99 months. A total of 16 cycles was performed in these 10 patients. Embryo transfers were canceled due to: failure to retrieve any oocyte at follicle puncture (n=3); lack of follicular growth (n=3); premature ovulation (n=1); and no fertilization achieved (n=1). Embryo transfers were performed in the remaining 8 cycles. Median number of oocytes retrieved in women with POI was 1 (0-2) and the mean number of metaphase 2 oocytes was 0.929±0.82. A total of eight grade 1 and 2 embryos were obtained and transferred. None of embryo transfers resulted in pregnancy. Cycle characteristics of women with POI following ovarian PRP injection is given in Table 1.

Ovarian PRP injection was performed in 50 poor responder women. Following PRP injections, 84 controlled ovarian stimulations were performed in those patients. Cycle outcomes before and after PRP injections were compared. Total gonadotropin doses required and days of stimulation were found to be significantly lower in cycles after PRP injection (p=0.006 and p=0.002, respectively). The number of retrieved oocytes (1.50±1.36 vs 2.18±1.66), number of M2 oocytes (1.16±1.06 vs 1.71±1.32), number of 2PN (0.84±0.89 vs 1.24±1.06), number of cleavage stage embryos (0.50±0.54 vs 1.04±0.96) and rate of top quality (grade 1) embryos obtained [7 (29.2%) vs 32 (59.3%)] were significantly higher in cycles following PRP injection (p=0.026, p=0.02, p=0.029,

p=0.001 and p=0.026, respectively). Frozen-thawed embryo transfers were performed in seven pre-PRP cycles and in 11 post-PRP cycles. Frozen-thawed embryo transfer rates were similar in pre- and post-PRP cycles (14% vs 13%, p=0.872). Cancellation rate of embryo transfer was significantly lower in cycles following PRP injection (p=0.03). One clinical pregnancy was identified in the cycles before PRP injection but resulted in miscarriage. Seven clinical pregnancies were identified in cycles after PRP injection and three of them resulted with miscarriage. There were no live births in pre-PRP cycles but there were four live births in post-PRP cycles. No significant difference was found in live birth rates among pre- and post-PRP cycles (0% vs 4.7%, p=0.296). Comparison of cycle outcomes before and after ovarian PRP injection is summarized in Table 2.

Outcomes of cycles performed in poor responders after PRP injection were subjected to a subgroup analysis stratified by time interval between PRP injection and initiation of the cycle. All of the clinical pregnancies and live births in our study population were achieved in patients when ovarian stimulation cycles commenced within 90 days following PRP injection (Table 3). Gonadotropin requirements tended to decrease in cycles initiated within the first 90 days following PRP injections. However none of these findings were statistically significant. Stratification of cycle outcomes with respect to interval between cycle starting day and PRP injection is given in Table 3.

Discussion

In this study IVF cycles were evaluated following ovarian PRP injection in patients with POI and poor ovarian response. The main outcome measure was live birth rate while other main cycle outcomes were also assessed.

Table 1. Outcomes of IVF cycles in patients with POI following ovarian PRP injection

Number of cycles	16
Median estradiol levels (pg/mL)	265 (59-894)
Median progesterone levels (ng/mL)	0.45 (0.1-1.5)
Median endometrial thickness (mm)	8.2 (7.2-9.5)
Median number of retrieved oocytes	1 (0-2)
Mean metaphase 2 oocytes	0.93±0.82
Fertilization rate	0.77±0.72
Number of day 3 embryo transfers	8
Number of grade 1 embryo	3 (37.5%)
Number of grade 2 embryo	5 (62.5%)
Mean number of transferred emryos	0.43±0.62

IVF: In-vitro fertilization, POI: Premature ovarian insufficiency, PRP: Platelet rich plasma

In poor responder women significantly increased numbers of oocytes, M2 oocytes, 2PN embryos, grade 1 embryos and cleavage stage embryos were obtained from cycles following ovarian PRP injection. These findings are consistent with previous studies (18-20). Although the effective mechanisms are not clear, it has been suggested that these findings may be due to the effect of platelet-derived cytokines which may improve the ovarian microenvironment, enhance ovarian vascular activation and stabilization or even result in de novo oocyte development from precursor stem cells (21-23).

Some case series and studies have reported pregnancies in women with POI following ovarian PRP injections, either spontaneously or via IVF (20,24-26). However, in the present study no live births occurred in women with POI after ovarian PRP injection. There was an increasing trend in live births following PRP injections in women with poor response but this increase was not significant, which again is in line with the studies conducted by Melo et al. (18) and Stojkovska et al. (27). This might be due to small sample sizes. However, in a previous study, general cumulative live birth rates were estimated to be approximately 13.7% in poor responders after two IVF cycles without PRP injections and this rate ranged between 4.4% and 17.2% when patients were stratified with respect to age (28). For poor responder women, live birth delivery rate following PRP injection was estimated as 4.7% in our study, lower than the reported cumulative live birth rates in poor responders as a whole in earlier studies. There does not seem to be any increase in live birth rate in poor responders when using ovarian PRP injection following the technique we used, possibly due to specific preparation techniques on the composition and thus the resultant effects of the PRP preparations. Different centrifugation processes are known to change the final composition of PRP solutions. For example, forces applied to samples exceeding 800 g in centrifugation has been shown to decrease the concentration of TGF-β in PRP preparations by disruption of platelets and granules containing growth factors (29). TGF-β mediates follicular development through effects on cellular differentiation, proliferation and chemotaxis and activation of various regulatory proteins (21). In animal models, inhibition of TGF-β pathways have been shown to reduce fertility by disrupting multiple ovarian processes, such as follicular development and cumulus-oocyte complex expansion and provokes premature luteinization of granulosa cells leading to ovulation failures (30,31). High TGF-β concentration in orthopedic studies is associated with bone deterioration and fibrocartilage calcifications (32). In the present study the centifugal force was equivalent to 1500 g, in accordance with PRP kit manufacturer's instructions. It should be noted that PRP preparation techniques that are suitable for extra-ovarian

applications might not be optimal for ovarian injection. Further research is needed in this area.

The effects of PRP preparations are entirely dependent on their exact composition. The presence of different proportions of other leukocytes, all of which are capable of secreting a broad range of cytokines, such as VEG-F and other proteins and may directly induce platelet degranulation (33). The protein contents of platelet granules may be both pro- and anti-inflammatory. Inhibition of the nuclear factor-kappa b (NF-kb) pathway by platelets is associated with suppression of inflammation and this effect is more prominent in leukocyte-poor rather than leukocyte-rich PRP preparations (34).

There are a wide range of variables that may affect the final composition of PRP preparations, including the donor hematological status and preparation technique. Weibrich et al. (35), using an animal model, demonstrated that PRP preparations with platelet concentrations between 1-6 fold of the donor whole blood platelet count enhanced peri-implant bone regeneration. This effect disappeared when the final PRP platelet count was either <1 or >6 times the whole blood platelet count. A study by Sills et al. (36) in reproductive medicine showed that the increase in anti-mullerian hormone levels in women following ovarian PRP injection was greater in women with higher whole blood platelet counts.

Whether the observed effects after PRP injection is a consequence of ovarian trauma caused by procedure is a matter of debate. The hippo signaling pathway is a tumor suppressor cascade that regulates cell proliferation, apoptosis and stem cell regeneration and is known to impede folliculogenesis by preventing progression of pre-antral follicles

Table 2. Comparison of outcomes of IVF cycles applied before and after ovarian PRP injection in poor responder patients

	Cycles before ovarian PRP injection	Cycles after ovarian PRP injection	p
Number of cycles	50	84	-
Total dose of gonadotropin (IU)	3907.5±990.15	3507.14±1076.94	**0.006**
Mean days of stimulation	10.76±1.83	9.73±1.82	**0.002**
Fertilization rate (2 pronuclear embryo/M2 oocytes)	42/58 (0.724)	104/144 (0.722)	0.976
Implantation rate (gestational sacs/transferred embryo)	1/28 (3.6%)	7/79 (8.8%)	0.357
Mean estradiol levels (pg/mL)	384.08±227.22	589.40±449.17	**0.014**
Mean progesterone levels (ng/mL)	0.62±0.49	0.60±0.48	0.786
Mean endometrial thickness (mm)	8.38±1.53	8.44±1.42	0.487
Mean number of retrieved oocytes	1.50±1.36	2.18±1.66	**0.026**
Mean number of metaphase 2 oocytes	1.16±1.06	1.71±1.32	**0.020**
Mean number of 2 pronuclear embryos	0.84±0.89	1.24±1.06	**0.029**
Mean number of cleavage stage embryo	0.50±0.54	1.04±0.96	**0.001**
Number of day 3 embryo transfers	21 (87.5%)	48 (85.7%)	1
Number of day 5 embryo transfers	3 (12.5%)	8 (14.3%)	
Mean number of transferred embryos	0.56±0.64	0.94±0.78	**0.006**
Number of grade 1 embryos	7 (29.2%)	32 (59.3%)	**0.026**
Number of grade 2 embryos	17 (70.8%)	22 (40.7%)	
Clinical pregnancies %, (n)	2% (1)	8.3% (7)	0.16
Cancellation rate %, (n)	52% (26/50)	33% (28/84)	**0.03**
Live birth delivery rates	0% (0/50)	4.7% (4/84)	0.296

IVF: In-vitro fertilization, PRP: Platelet rich plasma, M2: Metaphase 2

Table 3. Distribution of cycle outcomes due to interval between commencement and PRP injection

Interval between PRP injection and cycle initiation	<30 days	30-60 days	60-90 days	>90 days	p
Number of cycles	13	29	33	9	-
Gonadotropin doses required (IU)	3848.1±1908.54	3587.0±1033.3	3243.2±700.72	3725.1±685.3	0.427
Clinical pregnancies	0	4	3	0	0.696
Live births	0	2	2	0	0,724

to early antral follicles (37). This pathway is involved in a cell-contact type inhibition and polymerization of globular actin to filamentous actin inactivates the hippo signaling pathway (3). In light of this investigations into IVA techniques have resected, fragmented and re-transplanted ovaries in the presence of hippo inhibiors, protein kinase B (Akt) stimulators or by experimental direct trauma to disrupt the hippo pathway, with some success (3,5,6,11). Zhang et al. (4) conducted a study to observe the effects of ovarian biopsy and scratching on ovarian function. They took a 5 mm biopsy and inflicted three superficial scratches of 2-4 mm on each ovary. The observed improvement in ovarian functions were less than in IVA studies and the authors suggested that this may be due to insufficient disruption of hippo pathway, posibly due to insufficient ovarian trauma. Thus it is doubtful that inserting a 17G needle will inflict adequate damage to the ovary to disrupt the hippo pathway. The Yes-associated protein/transcriptional co-activator with PDZ binding motif (YAP/TAZ) system is an oncogenic component of the hippo pathway and its activation stimulates follicular growth (3). This system is regulated by mechanical factors. The YAP/TAZ system is activated by increased tensile forces within the cytoplasm and inhibited by decreased tensile forces (38). The exact mechanical forces applied on follicles that occur when injecting a fluid bolus into ovarian stroma, as well as its effects on the YAP/TAZ system, are hard to predict. Placebo-controlled trials involving ovarian PRP injections are lacking. However, the findings of Sills et al. (36) showed a correlation between patients' platelet counts and ovarian functions after PRP injections and this finding indicates at least some effects of ovarian PRP injection are not solely results of mechanical effects of injection.

Currently, PRP preparation techniques for ovarian PRP injections lack standardization. A wide range of PRP preparation techniques have been used in published studies, often without giving fine detail. In addition, final PRP preparations are also dependent on the hematological status of the donor women. Lack of standardization of these preparations means that comparison between studies is unreliable. Many PRP classification systems have been proposed to provide uniformity but none have been widely accepted (39). Among these, Magalon et al. (40) described a comprehensive classification system, the "DEPA classification", that has the advantage of retrospective application. However, to use DEPA precise cell counts for whole blood and the final PRP preparation should be known, together with volume of collected blood and injected PRP volume. When using commercial PRP preparation kits some of these data are not readily available without manufacturer co-operation. Rossi et al. (39) suggested that an ideal classification for PRP preparations to provide a degree of reproducibility and uniformity should include at least platelet counts, leukocyte count (with percentage of neutrophils), red blood cell count and concentration and dose of PRP preparation used. A limitation of the present study is the lack of these data. Apart from molecular research, inclusion of these parameters in future studies would help standardization and comparability of studies.

To date there is no consensus about optimal timing for intiation of IVF cycles following ovarian PRP injections. In the present study, IVF outcome was assessed in relation to the period between PRP injection and cycle initiation. There was a non-significant trend in required gonadotropin doses in cycles commenced within 90 days of PRP injection, with the lowest doses in cycles initiated between 60-90 days after PRP injection. Although there is no direct quantification of ovarian reserve, lower gonadotropin dose might suggest improved ovarian functions, peaking between 60-90 days after PRP injections. Earlier studies showed improved results of tests of ovarian reserve following PRP injection and it was suggested that the effect of PRP injection may be to enhance pre-antral follicular growth or prevent their atresia (18,25,36). Besides hormones and other gonadotropins, some as yet poorly understood paracrine factors are shown to regulate ovarian folliculogenesis. One of these is growth differentiating factor-9 (GDF-9). GDF-9 is an oocyte-derived local factor that is thought to act synergistically with bone morphogenetic protein-15 (BMP-15) to stimulate follicular development. GDF-9 enhances follicular growth beyond pre-antral stages of follicles and it is known to be secreted throughout folliculogenesis (37). Both GDF-9 and BMP-15 are members of TGF-β super family and their actions are known to overlap with other members of this group of proteins (41). There is evidence that GDF-9 stimulates progression of primary follicles to small pre-antral follicles (42). Under physiological conditions, progression of primary follicles to pre-antral follicles takes approximately 120 days (43). However supra-physiologic local ovarian TGF-β levels after PRP injection might hasten this process or trigger the shift from primary to small pre-antral follicles. Besides stimulation of pre-antral follicle growth, an increased number of hormone-responsive pre-antral follicles could be one of the possible reasons of reduced gonadotropin requirements observed in our study.

Moreover triggering of the shift from primary to pre-antral follicles might explain the delayed effects of PRP that were observed two to three months after injection, long after the degradation of injected cytokines. However there are still many uncertainties concerning the paracrine regulation of folliculugenesis, as well as in the composition of PRP.

Platelets are known to contain more than 800 types of proteins and more than 30 types of bioactive molecules that could be released into PRP preparations at various rates and concentrations upon degranulation or degradation (25,44).

One of the aims of future research in this field should be to identify which of these proteins and at what doses actually benefits outcome. In this way, a procedure which currently consists of the injection of a non-standardized soup of pro- and anti-inflammatory cytokines, differently affecting various target tissues may evolve into groundbreaking therapies.

Study limitation

Some limitations should be noted. This study lacked a control group. Cycle outcomes were compared in the same group of poor responder women before and after PRP injections. Therefore one should keep in mind the "regression to the mean" bias when interpreting our results. Larger studies with control groups would provide more precise data.

There are no reports of any serious adverse effects associated with ovarian PRP injections and no adverse side-effects were observed in our cohort. However, it should be noted that long term effects of this procedure are not known and administering highly concentrated growth factors to tissues carries the theoretical risk of inducing malignant transformation.

Conclusion

Intra-ovarian PRP injections do not appear to increase live birth rates or clinical pregnancy rates in poor responder women, at least using the techniques described herein. The heterogeneity of current methods used in the literature and inadequate understanding of paracrine mechanisms involved in folliculogenesis are barriers to improvement of this therapy. Further research is required to improve outcomes of intra-ovarian PRP injections.

Ethical Committee Approval: *Ethical approval for this study was obtained from Üsküdar University Faculty of Medicine at 28/05/2021 (approval number: 61351342/MAY 2021-04, date: 28.05.2021).*

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Brenner tumors of the ovary: clinical features and outcomes in a single-center cohort

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Abstract

Objective: The purpose of the present study was to evaluate the clinical and pathological features and oncological outcomes of Brenner tumors (BT).

Material and Methods: Evaluation was performed on the data of 46 patients with BTs retrieved from the oncology clinic database and pathology reports between 2005 and 2020.

Results: The median (range) age of the patients was 52 (22-75) years. Median (range) tumor size was 52.5 (5.0-300) mm. The tumor was benign in 37 (80.4%), borderline in one (2.2%), and malignant in the remaining eight (17.4%). Ten (21.7%) of the tumors were detected incidentally. Mixed tumor, BT plus another ovarian pathology, was found in 13 (28.2%). Recurrence developed in 2/8 (25%) with malignant BT (MBT). The stage of these patients was 3C, and both received chemotherapy after surgery.

Conclusion: BTs are rare and generally detected incidentally. MBTs are treated in the same way as epithelial tumors. Due to the rarity of these tumors, lymphadenectomy and optimal chemotherapy regimens are controversial issues. (J Turk Ger Gynecol Assoc 2022; 23: 22-7)

Keywords: Brenner tumors of the ovary, malignant Brenner tumors, mixed tumors, rare tumors

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Introduction

Ovarian Brenner tumors (BTs) are a rare type of epithelial ovarian tumor and constitute only 2-3% of all ovarian tumors (1). They were first described and named by Fritz Brenner in 1907 (2). BTs occur incidentally and frequently with other epithelial neoplasms (3). Incidental BTs are more common in oophorectomy specimens although, as the diagnosis is difficult, true incidence cannot be assessed (4).

The aim of this study was to report 46 cases with BTs of the ovary and to analyze the clinical and demographic features, and oncological outcomes.

Material and Methods

A retrospective evaluation was performed on patients with BT treated in our institution between 2005 and 2020. The clinical, surgical, and pathological data of the patients were collected from the gynecologic oncology department electronic database system, patient files, pathological reports, and operation notes. Data including age, menopausal status, tumoral features (tumor size, bilateral/unilateral), tumor markers (CA-125), surgical indications, type of surgical procedure, concomitant pathology, malignancy status, and follow-up information were obtained from the hospital registry. Written informed consent

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was obtained from all patients on admission for medical information to be used anonymously for academic purposes. Approval for the study was granted by the University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Care, Training and Research Hospital (approval number: 90057706-799/8, date: 30.10.2019).

Patients with malignant BT (MBT) and BT accompanied by another gynecological malignancy were included in the study, and post-surgical follow-up was performed every three months for the first two years, every six months for the following three years, and annually for the subsequent five years. The 2014 International Federation of Gynecology and Obstetrics (FIGO) staging criteria were considered. For patients treated before 2014, cancer staging was re-assessed using the FIGO 2014 system from surgical and pathological reports.

Gynaecological examination, abdominal ultrasonography, and measurements of CA-125 levels were routinely performed at each follow-up visit. Patients with borderline pathology were followed-up annually.

Statistical analysis

Data obtained in the study were analyzed statistically using SPSS, version 17.0 software (SPSS Inc., Chicago, IL, USA). The demographic data of the patients and disease characteristics were evaluated with descriptive statistics, with continuous variables reported as median, minimum-maximum values, and categorical variables as number and percentage (%).

Results

Evaluation was performed on 46 patients who presented during the study period. The patients had a median (range) age of 52 (22-75) years. The median (range) tumor size was 52.5 (5.0-300) mm. The median (range) preoperative CA-125 level was 19 (4.9-215) IU/mL.

Tumors were bilateral in 3 (6.5%) patients, unilateral in the right ovary in 21 (45.7%), and unilateral in the left ovary in 22 (47.8%). Twenty-five (54.3%) patients were postmenopausal. The tumor was benign in 37 patients (80.4%), borderline in 1 (2.2%) and malignant in 8 (17.4%).

The most frequent features leading to diagnosis were adnexal mass (71.7%), then myoma uteri (7%), followed by abdominal pain, abnormal uterine bleeding, and prolapse.

Tumours were detected incidentally during surgery for other indications in 10 (21.7%) cases. These were: cervical cancer (n=2); ovarian cancer (n=2); serous ovarian cancer (n=1); endometrioid type of ovarian cancer (n=1); myoma uteri (n=3); prolapse (n=1); high-grade cervical intraepithelial lesion (n=1); and endometrial cancer (n=1).

The patient with borderline BT accompanied by hyperplasia was found to have endometrial atypia, which was determined in preoperative endometrial biopsy and postoperative pathology.

Mixed tumors consisting of BT and another ovarian pathology were detected in 13 (28.2%) cases. Mucinous cystadenoma were concomitant in 7 (15.2%) patients, serous cystadenoma in 2 (4.3%), endometrioma in 2 (4.3%), struma ovarii in 1 (2.1%) and mature cystic teratoma in 1 (2.1%). The clinical and pathological features of the patients are presented in Table 1.

The median age was 52 years (range, 36-57 years) in cases with MBT and 52 years (range, 22-74 years) in benign cases.

Eight cases with MBT were examined separately in detail. Stage IIIC was identified in 4 patients, 1A in 1 patient, IIA in 1 patient, IC1 in 1 patient, and IC3 in 1 patient. The median (range) follow-up time was 75 (36-75) months. In this period, recurrence was observed in 2/8 (25%). The patient with recurrence at stage 3C, case no: 43, underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic lymph node dissection, appendectomy, and omentectomy, followed by six cycles of paclitaxel and carboplatin treatment, and had a recurrence in paraaortic + pelvic lymph node regions and the liver 86 months later. The other patient with recurrence (case no: 46) underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic-paraaortic lymphadenectomy, and total omentectomy due to adnexal mass. After six cycles of chemotherapy (cisplatin + paclitaxel), the patient showed pulmonary + liver + pelvic recurrence in the 13th month. The patient underwent bleomycin + etoposide + cisplatin chemotherapy and pelvic radiotherapy (RT), but died after 53 months due to progressive disease. The clinical and oncological characteristics of the cases with MBT are given in Table 2. The recurrences were detected by imaging.

Discussion

Tumors originating from the surface epithelium of the ovary are the most common ovarian neoplasms. BTs are a rare subtype of epithelial ovarian tumors. The WHO categorizes BTs into three types - benign, borderline, and malignant. BTs are known as transitional cell tumors because of their histological similarity to the urothelium resembling epithelial components (5).

BTs usually present in the fifth to sixth decades of life. In a series of 13 cases reported by Gezginç et al. (6), 61.5% of patients were post-menopausal and the median age was 55.6 years. Green et al. (7) also reported the mean age to be 58 years in 22 patients. Of the 46 patients in the current series, 54.3% were postmenopausal, and the median age was 52 years. The vast majority of reported cases of BT consist of small tumors and are detected incidentally when oophorectomy is performed for some other indication. In these cases tumor size is small, usually <2 cm and most patients are asymptomatic (4).

Table 1. The Clinical and pathological features of the patients

N	A	M	Side	Size/mm	Presenting symptom	Concomitant pathology	Surgery	CA-125	Histology
1	57	+	R	33	Adnexal mass-ovarian cancer	Serous ovarian carcinoma	Tah + Bso + Bpplnd + App. + Omm.	215	Benign
2	55	+	R	N/A	Cervical cancer	Cervical cancer	Type 3 hysterectomy + Bso + Bpplnd	N/A	Benign
3	55	+	R	50	AUB	-	Tlh + Bso	6.2	Benign
4	42	-	L	N/A	Recurrent cervical cancer	Cervical cancer	Pelvic exenteration	N/A	Benign
5	46	-	L	N/A	Endometrial cancer	Endometrial cancer	Tlh + Bso	N/A	Benign
6	43	-	L	150	Adnexal mass + Abd. pain	Mucinous cystadenoma	Left uso	10.9	Benign
7	65	+	L	120	Adnexal mass + Abd. pain	-	Tah + Bso	N/A	Benign
8	54	+	R	20	HSIL surgical margin +	HSIL surgical margin +	Tlh + Bso	N/A	Benign
9	49	-	L	150	Adnexal mass + AUB	Mucinous cystadenoma	Tah + Bso + App.	13	Benign
10	53	+	L	20	Adnexal mass-ovarian cancer	Endometrioid ovarian carcinoma	Type 2 hysterectomy + Bso + Bpplnd + App. + Omm.	32	Benign
11	52	+	L	50	Adnexal mass	-	Tah + Bso	16	Benign
12	57	+	B	50	Adnexal mass	-	Tlh + Bso	30	Benign
13	69	+	L	200	Adnexal mass	-	Tah + Bso	28	Benign
14	56	+	R	55	Adnexal mass	Struma ovarii	Tah + Bso	5	Benign
15	63	+	R	80	Adnexal mass	-	Tah + Bso	N/A	Benign
16	38	-	R	56	Adnexal mass	-	Right uso	9.4	Benign
17	74	+	R	40	Adnexal mass	Endometrioma	Tah + Bso	19	Benign
18	73	+	L	100	Adnexal mass + AUB	Mucinous cystadenoma	Tah + Bso	40	Benign
19	50	-	L	200	Adnexal mass	Mucinous cystadenoma	Right uso + Left salpingectomy + App.	100	Benign
20	43	-	L	55	Adnexal mass	Mature cystic teratoma	Left uso	77	Benign
21	47	-	R	85	Adnexal mass	Mucinous cystadenoma	Right uso	12	Benign
22	48	-	R	40	Adnexal mass	Endometrioma	Tah + Bso	37	Benign
23	48	-	R	45	Myoma uteri	-	Tah + Bso	N/A	Benign
24	54	+	R	6	Myoma uteri + uterine prolapse	-	Tah + Bso	N/A	Benign
25	34	-	L	60	Adnexal mass	Mucinous cystadenoma	Cystectomy	9.2	Benign
26	54	+	R	15	Adnexal mass	-	Tah + Bso	13	Benign
27	60	+	R	5	Uterine prolapse	-	Tah + Bso	6	Benign
28	53	+	L	30	Myoma uteri	-	Tah + Bso	N/A	Benign
29	52	+	L	65	Adnexal mass	Mucinous cystadenoma	Left uso	4.9	Benign
30	50	+	L	10	Myoma uteri	-	Tah + Bso	6.3	Benign
31	46	-	R	40	Adnexal mass	-	Right uso	8.4	Benign
32	52	-	R	25	Myoma uteri	Serous cystadenoma	Tah + Bso	N/A	Benign
33	52	-	R	25	Myoma uteri	-	Tlh + Bso	24	Benign
34	39	-	L	40	Adnexal mass	Serous cystadenoma	Left uso	24.4	Benign
35	22	-	L	45	Adnexal mass	-	Cystectomy	9.5	Benign
36	59	+	R	8	Myoma uteri	-	Tah + Bso	N/A	Benign
37	50	-	L	270	Adnexal mass	-	Tah + Bso	152	Benign

Table 1. Continued

N	A	M	Side	Size/mm	Presenting symptom	Concomitant pathology	Surgery	CA-125	Histology
38	70	+	L	100	Adnexal mass + AUB	Atypical hyperplasia	Tah + Bso	38	Borderline
39	75	+	B	36	Adnexal mass	Breast cancer history	Tah + Bso + Bpplnd + App. + Omm.	20	Malignant
40	57	+	L	55	Adnexal mass	-	Tah + Bso + Omm.	N/A	Malignant
41	48	-	L	200	Adnexal mass	-	Tah + Bso + Bpplnd + App. + Omm.	9.6	Malignant
42	37	-	R	300	Adnexal mass	-	Tah + Bso + Bpplnd + App. + Omm.	12	Malignant
43	49	-	R	N/A	Adnexal mass	Mucinous cystadenoma	Tah + Bso + Bplnd + App. + Omm.	N/A	Malignant
44	75	+	R	N/A	Adnexal mass	-	Tah + Bso + Bpplnd + App. + Omm.	95	Malignant
45	36	-	R	180	Adnexal mass	-	Tah +Bso + Bpplnd + App. + Omm.	209	Malignant
46	55	+	B	150	Adnexal mass + Abd. pain	-	Tah + Bso + Bpplnd + App. + Omm.	64	Malignant

N: Patient no, A: Age, M: Menopausal status, AUB: Abnormal uterine bleeding, Abd.pain: Abdominal pain, Uso: Unilateral salpingo-oophorectomy, Tah: Total abdominal hysterectomy, Bso: Bilateral salpingo-oophorectomy, Tlh: Total laparascopic hysterectomy, App: Appendectomy, Bpplnd: Bilateral pelvic-paraaortic lymph node dissection, HSIL: High grade squamous intraepithelial lesion, L: Left, R: Right

Table 2. The clinical and oncological characteristics of cases with malignant Brenner tumors

Case no	Age	Stage	Chemotherapy	Recurrence (time/site/treatment)	Follow-up time (m)	Outcome
39	75	IIIC	C + PTx (6 cyc.)	No	47 m	Ned/Alive
40	57	IIA	C + PTx + RT (7 cyc.)	No	12 m	NA
41	48	IA	-	No	96 m	Ned/Alive
42	37	IC1	C + PTx (6 cyc.)	No	115 m	Ned/Alive
43	49	IIIC	C + PTx (6 cyc.)	86 m (paraaortic + pelvic lymph node and liver) Surgery + 6 cyc. C + PTx	96 m	Ned/Alive
44	75	IIIC	C + PTx (6 cyc.)	No	12 m	DOD
45	36	IC3	C + PTx (6 cyc.)	No	125 m	Ned/Alive
46	55	IIIC	Cis + PTx (6 cyc.)	13 m (pulmonary + liver + pelvic side) BEP + pelvic RT	53 m Progressive disease	DOD

Cyc: Cycle, C: Carboplatin, PTx: Paclitaxel, Cis: Cisplatin, RT: Radiotherapy, BEP: Bleomycin + Etoposid + Cisplatin, Ned: No evidence of disease, DOD: Dead of Disease, NA: Not available, m: Month

In the current study, 10 cases were detected incidentally after surgery for other indications. In these cases, the size of the tumor varied from 5 mm to 45 mm (Table 1).

BTs can be accompanied by mucinous cystadenoma, serous cystadenoma, benign cystic teratoma, or struma ovarii in approximately 20% of cases (8). Similarly, in the current study, coexistence with benign ovarian tumors was detected in 13 (28%) cases. Roma and Masand (9) reported that up to 27% of BTs were associated with mucinous tumors. The coexistence of struma ovarii and BT is rare. According to the current literature, only seven cases have been reported (10). The origin of the BT and the struma ovarii association may be the germ-cell, as described in various studies, or due to the metaplastic features of the BT (10,11).

BTs might be accompanied by other ovarian tumors and be associated with endometrial pathologies in 4-14% of patients. The stromal component of the BT, resembling the theca cells of the ovary, produces estrogen, which may be related to estrogen-related pathologies (3). In the current cohort, BTs were seen to coexist with atypical endometrial hyperplasia in one patient and endometrioid-type endometrial cancer in another.

Synchronous tumors of the female genital tract account for only 1-6% of all genital neoplasms (12). Similarly, in this study, one case was diagnosed incidentally in a case of serous ovarian carcinoma and one case in an endometrioid ovarian tumor. Coexistence with the endometrioid ovarian tumor and the history of breast cancer in one patient also supports estrogen-related events. These findings also explain the vaginal bleeding complaint in these patients. Although no data exist about the coexistence of cervical cancer and BTs in the literature, two (4.3%) patients had cervical cancer in the current study. This might have resulted from the fact that the study was conducted in a gynecological oncology clinic.

BTs are known to range from benign to malignant. In the current study, 1 patient had borderline and eight patients had MBT. Borderline BTs are rare and defined as "epithelial proliferation without stromal invasion" and only 60 cases have been published in the English literature to date (13). Most of the cases in the literature were reported as older than 50 years. Presenting with postmenopausal bleeding indicates that some of the borderline BTs may contain hormone-secreting elements. The case in the current study with borderline BT was 70 years old and the main complaint was postmenopausal bleeding. Histopathological examination showed concomitant atypical endometrial hyperplasia. Similar to the cases in literature, this finding indicates that endometrial hyperplasia may have developed due to the hormonal effects of borderline BT.

Whereas the vast majority of BTs are benign and often found incidentally, MBT, accounting for <5% of all BTs, are extremely rare (5). The clinical and oncological features of the eight patients with MBT are summarized in Table 2. The median age of the MBT cases was 52 years, similar to the study of Han et al. (14). A small number of studies provide the only available information about the treatment of these patients, and the optimal adjuvant management remains unclear. Surgery is the main treatment, as in the case of other epithelial ovarian carcinomas. In the reported case series carboplatin and paclitaxel had been used for adjuvant chemotherapy, as in other epithelial ovarian tumors (6,14). In the presented series, all patients, except one case (stage 1A), received paclitaxel-carboplatin as adjuvant therapy in line with previous reports. A recent large retrospective study reported the median tumor size as 10 cm for MBT and most of these were unilateral (15). In this case series, the median tumor size was 16.5 cm and the majority of the tumors (6/8) were unilateral.

Lymph node dissection is a controversial issue in MBT. Nasioudis et al. (15) reported that lymphatic spread and lymph node dissection did not confer any disease-specific survival (DSS) benefit to these patients. Approximately 50% of patients with surgical tumor excision had concomitant lymph node dissection, but only 5% of these patients had evidence of lymphatic spread. In that study, no DFS difference was found between the lymphadenectomy group and non-lymphadenectomy group (15). In the current study, lymph node dissection was performed in all except two patients (stage 1A and IIA). No recurrence was observed in these early stage patients.

Complete chemotherapy response was obtained from 7/7 patients who received carboplatin + paclitaxel chemotherapy in this series. Similarly, Gezginç et al. (6) reported a complete response rate in 9/10 patients, and the recurrence rate was 7/10. These results support the importance of complete cytoreductive surgery before chemotherapy. In the current study recurrence was seen only in 2/8 MBT patients. One of the patients with recurrence was given chemotherapy following surgery for recurrence, and that patient is currently alive without disease. The second patient, who had recurrence after primary adjuvant chemotherapy was given bleomycin, etoposide, and cisplatin. Palliative RT was given for progressive disease and the control of pelvic recurrence. The patient died from the disease in the 53rd month. NCCN guidelines on epithelial ovarian cancers do not include RT as a primary treatment recommendation, but reference palliative RT for local symptom control (16).

Although specific tumor markers for MBT have not been identified, CA-125 can be used to monitor the effectiveness of therapy and to detect recurrence during follow-up (17). In the current study, 3/8 patients (38%) had CA-125 levels >35 IU/mL. Roth et al. (18) reported that MBTs are associated with better survival compared to other epithelial ovarian tumors. In the current study, 4/8 patients were diagnosed at stage IIIC and the others were stage IA, IIA, IC1, and IC3. Two reported recurrences were seen at stage IIIC. In the early stages, no recurrence was observed. These findings support the suggestion that DSS is better in the early stages, in agreement with the findings of Nasioudis et al. (15).

This report presents a single-center experience over fifteen years. Due to the relatively low number of cases, the cohort provides information about benign, borderline, and malignant MBTs of the ovary. This study can be considered to provide valuable information in terms of oncological results about MBTs, as rare case reports and a limited number of case series in many reports are presented together.

Conclusion

BTs are rare and mostly incidental findings. It should be remembered that these tumors can secrete hormones and can cause endometrial pathologies. Especially for malignant forms, multicenter studies are needed to be able to establish the optimal treatment regimen and surgery.

Ethics Committee Approval: *This study was approved by the University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Care, Training and Research Hospital, approved the study (approval number: 90057706-799/8, date: 30.10.2019).*

Informed Consent: *Written informed consent was obtained from all patients on admission for medical information to be used anonymously for academic purposes.*

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Retrospective clinical evaluation of indications for termination of pregnancies due to fetal anomaly

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Abstract

Objective: To assess the indications for termination of pregnancy (TOP) in pregnant patients who were followed up with suspicion of fetal anomaly in a Turkish tertiary referral center.

Material and Methods: This retrospective study was carried out in patients who were followed up with suspicion of fetal anomaly between May 2016 and May 2019 at the Perinatology Clinic of Obstetrics and Gynecology Department in Pamukkale University Hospital, which is a tertiary hospital in Denizli province in Turkey. Women were divided into two depending on gestational period: group 1 ≤22 weeks; and group 2 (>23 weeks of gestation).

Results: Four hundred and seventeen pregnant women were evaluated and TOP was performed at a mean gestational age of 27.7±6.3 weeks. There were 308 (73.8%) women in group 1 and 109 (26.2%) in group 2. The decision to terminate pregnancy was due to fetal anomaly in 117 (28.1%). The majority of termination pregnancies in group 2 were performed because of multiple malformations and/or central nervous system defects. All chromosomal diseases were detected in group 1.

Conclusion: With a good perinatal screening program, fetal anomalies can be diagnosed early. Therefore, early TOP is possible. Thus, pregnancy termination can be made before reaching the life limit. (J Turk Ger Gynecol Assoc 2022; 23: 28-32)

Keywords: Fetal anomalies, termination of pregnancy, perinatal diagnosis, congenital anomalies

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Introduction

At present improved assessment of fetal development and fetal anomalies during pregnancy can be performed using advanced transvaginal ultrasonography (USG) techniques and/or routine prenatal follow-up (1). Thus, more accurate information about fetal prognosis is obtained and better counseling can be given. In addition to USG examination, prenatal screening tests are also used for fetal anomaly screening (2). Measurements of the fetal nuchal translucency thickness by USG and double screening tests are used in the routine follow-ups between 11 and 14 weeks of gestation. The triple screening test combined with USG examination is performed at 16 and 18 weeks of gestation in each pregnant woman, who has not been able to do a double screening test earlier and who would like to have screening done. Based on the results of the double screening test, optional chorionic villus sampling (CVS) may be offered when screening has indicated a high risk of fetal anomaly. Alternatively, optional amniocentesis may be performed in women when triple screening indicates a high risk for fetal anomaly. Screening, diagnosis and management of fetal anomalies in pregnancy allows parents to come to terms with the situation and to perhaps plan for the future.

In many countries, termination of pregnancy (TOP) is regulated by law and can be performed without medical treatment due to fetal anomalies. TOPs are permissible, if required up to the 10th gestational week in Turkey since 1983 (3). However, medical evacuation may be performed electively after the 10th gestational week, either in case of serious fetus anomalies or a risk to the mother of serious incurable disease or death as a result of the continuation of the pregnancy. There is no upper limit of the gestational week for medical evacuation,

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and pregnancy can be terminated at any gestational week if there is a serious condition reported by two specialists. In many European countries, TOPs can be performed until term if the presence of serious or fatal fetal anomalies is confirmed. This is the situation in France, England, Wales, Belgium, Finland, Norway (under limited conditions) and Sweden (after approval by the National Board of Health and Welfare).

The aim of this retrospective study was to investigate and discuss the results of incidence and indications for TOPs in pregnant patients followed with suspicion of a fetal anomaly in Pamukkale University Faculty of Medicine, Department of Obstetrics and Gynecology, Perinatology Clinic.

Material and Methods

This study was approved by the Ethics Committee of Non-interventional Clinical Studies of Pamukkale University (approval number: 60116787-020/4318, date: 17.01.2018) and conducted retrospectively in accordance with the Helsinki Declaration. This study included data collected between May 2016 and 2019 from the Perinatology Clinic of the Obstetrics and Gynecology Department, Pamukkale University Hospital, a tertiary hospital of Denizli province in Turkey. Routine prenatal screening/diagnosis was performed in all pregnant women at 20-22 weeks of gestation, as per international and national guidelines. From these records, pregnancies when there was a suspicion of fetal anomaly were identified and included in the study. USG examination was performed using an Esaote MyLab Twice ultrasound diagnostic unit by a perinatology specialist (B.K.). Fetal echocardiography, fetal karyotyping and invasive prenatal tests, followed by genetic counseling were also recommended to patients when necessary. Moreover, a routine universal screening program for gestational diabetes mellitus was carried out for all patients.

Fetal anomalies were classified as congenital malformations, chromosomal abnormalities and genetic abnormalities. Congenital malformations were also defined into subgroups according to affected major organ systems. Pregnant women with a fetus having more than one system-related abnormalities were recorded as multiple anomalies (4).

After the examinations were completed, a decision concerning medical dilation and evacuation was made, taking into account the severity of the abnormality and likely seriousness of handicap, in conjunction with gynecology and obstetrics and related branch specialists. Counseling included information on the termination procedure, in addition to alternative management options. TOPs were performed after obtaining the consensus report which gave detailed information about the fetal anomaly and included parental approval. Oral or vaginal misoprostol induction, with or without oxytocin, was generally used as the main procedure after second-trimester TOP. The route, optimal dose and dosing intervals of misoprostol were chosen based on gestation week, obstetric history, clinical guidelines and practice (5-7). In addition, the need for additional oxytocin for cervical augmentation was determined, based on examinations and clinical conditions. Dilatation and curettage with vacuum aspiration were performed for removal of the retained product of conception due to incomplete or partial expulsion of the fetus and placenta. None of the delivered fetuses were alive. In case of failed induction by misoprostol administration or patients who have had three or more cesarean sections without any medical intervention, a hysterotomy operation was conducted.

All pregnant women with a single fetus who were examined at Pamukkale University Hospital due to suspected fetal anomalies were included in this study. Multiple gestations, unwanted pregnancies and patients whose fetus was not alive during hospital admission were excluded from the study. In addition, pregnant women with a single fetus diagnosed with prenatal fetal anomaly were also evaluated, and they were divided into two subgroups according to the gestational week at which termination was recommended. Also, pregnant women for whom termination was not recommended were divided into two subgroups according to the first examination week. Group 1 contained women with pregnancies up to and including the 22nd gestational week while group 2 included those from the 23rd gestational week onward. Medical records/obstetrical features and demographic information of the patients enrolled in the study were retrieved from the hospital information management system (Probel) and patients' follow-up files.

Statistical analysis

Data were evaluated with SPSS, version 20 (Statistical Package for the Social Sciences, IBM Inc., Chicago, IL, USA). Normality test was performed to apply the appropriate test. Numerical values are shown as mean ± standard deviation, or number and percentage (n, %). Chi-square test and Student's t-test were used in the analyzes. A p-value of 0.05 was assumed to indicate significance.

Results

In this study, a total of 417 pregnant women who were followed with suspected fetal anomalies between May 2016 and 2019 at the Perinatology Clinic of Obstetrics and Gynecology Department in Pamukkale University Hospital were included. During the study period 19,347 patients were examined and 2145 pregnant women delivered. The incidence of TOP in the clinic was 5.45 per 100 live births during the investigation period. The decision for TOP due to fetal anomaly was made in 117 pregnant women. Although detailed advice and information were given about the fetal anomalies, 24 (12.05%) parents

refused TOP and gave birth, of whom there were 18 in group 1 and six in group 2. Six of these pregnancies were longer than 22 weeks of gestation. Of the remaining 117 pregnancies, 105 were in group 1 (≤22 weeks) and 12 were in group 2 (≥23 weeks).

Demographic and clinical characteristics of patients are presented in Table 1. CVS was recommended to 33 (7.9%) pregnant women who were examined with suspicion of fetal anomaly and it was performed in 22 (5.3%). Amniocentesis was performed in one who was recommended CVS but refused it. Also, CVS was performed on one at 12 weeks of gestation and the TOP decision was taken at 23 weeks of gestation.

The mean age in those who terminated pregnancy was similar in group 1 (27.8±6.1) years and group 2 (27.1±8.1) years. Unsurprisingly, the mean gestational age in those who terminated pregnancy was higher in group 2 (24.5±1.3) than group 1 (16.5±3.6). The gestation age range at which TOP was conducted in group 2 was between 23 and 27 weeks. The majority of terminations were conducted between 11 and 22 weeks of gestation (89.74%).

The indications for TOP are presented in Table 2. The most common cause of termination in group 1 (≤22 weeks) was found to be central nervous system anomalies, multiple anomalies, cystic hygroma, cardiovascular system anomalies, chromosome anomalies, whereas it was multiple anomalies and central nervous system anomalies in group 2 (≥23 weeks). The distribution of central nervous system anomalies in group 1 was: spina bifida (n=11) (9.5%); anencephaly (n=8) (6.8%); hydrocephalus (n=6) (5.1%); holoprosencephaly (n=2) (1.7%); corpus callosum agenesis (n=1) (0.8%); and Dandy-Walker malformation (n=1) (0.8%). There were no cases of lung, face or skeletal system abnormalities that resulted in TOP. Trisomy 21 was the most common cause of chromosomal anomalies in group 1 (≤22 weeks), while no chromosomal anomalies were found in group 2 (≥23 weeks). The number of patients with chromosomal and genetic abnormalities was: trisomy 21 (n=5) (4.3%); trisomy 18 (n=2) (1.7%); triploidy (n=1) (0.8%); and thalassemia major (n=3) (2.5%).

In the present study, a vaginal termination induction was achieved (misoprostol with or without oxytocin induction and/or dilatation and curettage) in 114 patients (97.4%), while a hysterotomy was performed in three patients (2.6%). There was no significant maternal morbidity after TOP in the two groups. There were no patients with hysterectomy or uterine rupture in either group.

Discussions

The results of this study show that early diagnosis can be achieved by effectively detecting fetal anomalies before the 22nd gestational week. Thus, termination can be performed and thus minimizing the risk before the period of advanced pregnancy occurs. Furthermore, it was found that pregnant women were more likely to accept invasive procedures in the first weeks of pregnancy. As the pregnancy progressed, it was evident that these pregnant women became less likely to accept invasive procedures, possibly due to their maternal instincts. Early diagnosis of congenital abnormalities is also important for offering parents all choices, including TOP, both for ethical and legal reasons (8,9). Therefore, TOP is a critical decision that should be taken through a multidisciplinary committee with the parents involvement (10,11).

Similar to other studies, multiple malformations and central nervous system anomalies were detected more frequently at advanced gestational weeks (4,12). However, unlike in previous studies, chromosomal anomalies were detected at earlier gestational weeks in our study (13,14). In a study conducted by Aslan et al. (14) in 2007 in Turkey, chromosomal abnormalities were detected at advanced gestational weeks, while they were identified earlier in this study. In addition, and similar to previous studies, the most common chromosomal abnormalities were

Table 1. Demographic and clinical characteristics of pregnant women

Variables	Group 1 (n=308, 73.8%)	Group 2 (n=109, 26.2%)	p
Age (years)	29.2±6.5 (16-45)	26.9±6.2 (16-42)	0.2
Gestational age (weeks)	17.07±3.05 (11-22)	27.6±4.06 (23-38)	<0.01*
Termination (n)	105 (25.2%)	12 (2.8%)	<0.01*
CVS recommended (n)	32 (7.7%)	1 (0.2%)	0.006*
CVS performed (n)	22 (5.32%)	0	0.01*
Amniocentesis recommended (n)	125 (30%)	0	<0.01*
Amniocentesis performed (n)	47 (11.3%)	0	<0.01*
History of fetal anomaly (n)	11 (2.6%)	0	0.04*
Thalassemia (n)	12 (2.8%)	0	0.03*

Data are given as mean ± standard deviation (minimum-maximum) or count and percentage n (%).
*: p<0.05 statistically significant
CVS: Chorionic villus sampling

Table 2. The indications for TOP

	Group 1	Group 2	Total
Central nervous system	29 (24.9%)	6 (5%)	35 (29.9%)
Cystic hygroma	18 (15.3%)	0	18 (15.3%)
Cardiac system	12 (10.3%)	0	12 (10.3%)
Urinary system	3 (2.5%)	0	3 (2.5%)
Gastrointestinal system	3 (2.5%)	0	3 (2.5%)
Multiple anomalies	24 (20.5%)	6 (5%)	30 (25.5%)
Hydrops	3 (2.5%)	0	3 (2.5%)
Anhydramniosis	2 (1.7%)	0	2 (1.7%)
Chromosome anomalies	8 (6.8%)	0	8 (6.8%)
Genetic abnormalities	3 (2.5%)	0	3 (2.5%)
Total	105	12	117
Values are given as n (%), TOP: Termination of pregnancy			

detected by invasive prenatal tests and were trisomy 21 and trisomy 18 (12,14-16). In contrast to earlier studies, cardiac anomalies were found at earlier gestational weeks in our study, which is similar to the detection period of chromosomal anomalies (14-16). However, in later studies, there was a trend to detect cardiac anomalies at earlier gestational weeks, similar to the results of the present study (4,17). This result is thought to be due to wider use of fetal echocardiography and increased experience of fetal cardiac ultrasound examination amongst clinicians. Another reason for detecting chromosomal and cardiac anomalies in earlier gestational weeks may be a positive contribution of the family medicine system. Preventive family medicine system legislation was first introduced in 2004 in Turkey and full national coverage was available after 2010. The system of preventive family medicine provides a closer and more regular follow-up of pregnant women so this is most probably the cause of the difference between earlier studies and this one. In addition, it was found that pregnant women who had experienced fetal anomalies in an earlier pregnancies began early pregnancy follow-up with a concern about fetal anomaly.

In a study conducted by Raupach and Zimmermann (18), the most common causes of fetal anomaly in pregnant women were reported to be cardiac and skeletal system anomalies. In this study, skeletal system anomalies were detected in 2.6% of early gestational week pregnancies and were followed up without termination. Also, the reasons for misdiagnosis of fetal anomaly have been reported in the literature as unfavorable fetal position, oligohydramnios and multiple pregnancy (18).

In this study, 419 pregnant women were clinically followed due to fetal anomaly, 117 of them resulted in termination. In addition, despite clinical advice, 24 (12.05%) women chose to carry the pregnancy to term and deliver. A study from France reported that the proportion who did not accept pregnancy termination was between 6.6-15% (19). The reason for the increase in refusal of pregnancy termination can be explained by the increase in the number of surgical interventions and treatments that increase the survival chance of some fetal anomalies. In addition, fetal anomalies diagnosed in advanced weeks due to delaying of prenatal diagnosis at earlier gestational weeks may survive despite their anomalies due to improvements in newborn care support units and facilities. However, severe anomalies mostly lead to recurrent interventions and increased morbidity and mortality (10,20).

Study limitation

There are some limitations to this study. Since it was a retrospective study, data were obtained from patient records. Another limitation was the relatively small number of patients. There is no upper gestational week limit for TOP according to Turkish law. However, TOP after the 24th gestational week is considered to be unethical according to the Ankara Declaration of the Maternal-Fetal and Perinatology Society of Turkey in 2011 (8,21,22). Therefore, live birth after TOP in the viability zone may be a major problem if the indication is not ethically convincing. In this study, twelve patients (10%) were terminated after the 22nd gestational week because of central nervous system and multiple anomalies, taking into account legal and ethical factors and all 12 fetuses were delivered dead. Although the law permits TOP at any gestational age in Turkey, in practice there is an assumed upper limit for gestational age in terms of ethical termination which seems to be the main reason for this low rate of late TOP.

Conclusion

The establishment of systematic protocols to evaluate fetal organs and systems will be effective in detecting fetal anomalies at early gestational weeks. TOP may be performed after careful and detailed prenatal screening and diagnosis of fetal anomalies, but termination decisions may be affected by national laws, health system, parental education level, socioeconomic status, religious beliefs and cultural beliefs. A decision to terminate should be considered as a multidisciplinary decision with the parent, involving gynecologist and obstetrician, pediatric neurologist as appropriate.

Ethics Committee Approval: *The study was approved by the Ethical Committee of the Pamukkale University Faculty of Medicine (approval number: 60116787-020/4318, date: 17.01.2018).*

Informed Consent: *Retrospective study.*

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What is the optimum number of follicular flushes in mono-follicular in-vitro fertilization cycles in a poor responder population?

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Abstract

Objective: Assessment of the optimal number of follicular flushes on retrieval rate and quality of oocytes in mono-follicular in-vitro fertilization (IVF) cycles.

Material and Methods: A retrospective analysis of 246 oocyte pick-up procedures in mono-follicular IVF cycles of 226 poor responder women was performed. The primary endpoint was oocyte retrieval rate in the initial aspirate versus subsequent flushing episodes. The secondary endpoints were oocyte maturity, fertilization rates and embryo cleavage.

Results: The procedure was successful in 187 cycles (76%), of which 160 metaphase-II oocytes were retrieved. Retrieval rates were similar for natural and modified natural cycles (p=0.595). The initial aspirate provided 54% of the total yield and the rest was obtained from up to four episodes of flushing. Follicular flushing increased oocyte recovery rate from 41.1% to 76%. None of the oocytes retrieved after three flushes fertilized. Oocyte maturity, fertilization and embryo cleavage rates were comparable for oocytes from the initial aspirate and one or two episodes of flushing. Oocytes obtained after the third flushing episode developed into poor quality embryos.

Conclusion: Flushing confers a benefit for oocyte recover rates in mono-follicular IVF cycles in poor responder women. However, more than three attempts at flushing were not associated with good outcome. (J Turk Ger Gynecol Assoc 2022; 23: 33-7)

Keywords: Oocyte retrieval, in-vitro fertilization, assisted reproductive technologies

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Introduction

Since the early days of human in-vitro fertilization (IVF), ovarian stimulation cycles have gradually replaced natural cycles owing to the benefits of increased oocyte yield and improved pregnancy rate (1). Although natural cycle IVF has regained attention in parallel with the increased interest in minimal ovarian stimulation strategies, in many clinics it is considered as the last resort for women who do not respond to ovarian stimulation with more than a single follicle. Despite a bleak prognosis, a considerable number of women opt for multiple treatment attempts with their own oocytes before convincing themselves to proceed with oocyte donation and some others do not or cannot consider this option due to personal, religious, or legislative reasons.

The success of natural cycle IVF is impeded, however, by high cancellation rates because of premature ovulation, failed oocyte retrieval, and fertilization or cleavage problems (2). As the success is dependent on the retrieval of the oocyte presumed to be in the single growing follicle, flushing is commonly performed when the initial aspirate is negative. However, data regarding the benefit of flushing during oocyte retrieval is not conclusive and is mainly derived from stimulated cycles with multiple growing follicles (3,4). Excessive flushing is associated with long operative times and wasted flushing medium. Prompted by the scarcity of data, we retrospectively

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analyzed our mono-follicular IVF cycles to assess the optimal number of flushes.

Material and Methods

Study Population and Participants

This was a retrospective analysis of 279 oocyte pick-up (OPU) procedures performed in a tertiary care infertility center between January 2016 and December 2018 for natural (n=126) and modified natural (n=153) IVF cycles. Data regarding female age, body mass index (BMI), serum estradiol (E_2) level and diameter of the follicle at the time of ovulation trigger, number of flushes, oocyte maturity, fertilization and embryo quality were extracted from an electronic database. At the beginning of IVF treatment, all patients gave informed consent that their anonymized data to be used for research projects in the future. Treatment cycles of patients with more than one growing follicle (n=8) and premature ovulation (n=25) were excluded from the analysis. The study group included 99 natural and 147 modified natural IVF cycles.

During natural and modified natural IVF cycles, ultrasonographic monitoring was started on the second or third day of menstruation to exclude the presence of ovarian cysts that may be confused with a growing follicle. In the presence of a sonolucent structure >10 mm in size, serum estradiol was measured to differentiate between a growing follicle and a cyst. Ovulation was triggered with 250 μg of recombinant human chorionic gonadotropin (Ovitrelle®, Merck-Serono, Italy) when the mean follicle diameter reached or exceeded 16 mm. In modified natural IVF cycles, 75 IU recombinant FSH (Gonal F®, Merck-Serono, Italy) and gonadotropin-releasing hormone antagonist (Cetrotide®, Merck-Serono, Italy) was started when the follicle reached 12 mm in diameter. Ovulation was triggered with 250 μg of recombinant human chorionic gonadotropin (Ovitrelle®, Merck-Serono, Italy) when the mean follicle diameter reached or exceeded 16 mm. Indomethacin suppositories (Endol sup®, 100 mg, Deva, Turkey) were administered every 12 hours, starting with the ovulation trigger and continued until egg collection. Oocyte retrieval was performed under local anesthesia 34-36 hours after triggering ovulation, using a 17-gauge double-lumen needle (K-OPSD-1735-B-L, Cook, Australia), connected to a vacuum pump (K-MAR-5200, Cook, Australia). The aspiration pressure was set at 150 mmHg. The follicle was aspirated and an additional 1.5 cc (this is the volume of the aspiration tubing of the needle) of flushing medium was given and aspirated again to retrieve the oocyte-cumulus corona complex (OCCC) if trapped in the aspiration tubing. This was referred to as the initial aspirate. If no OCCC was observed, flushing was affected using a specifically formulated medium (ASP, Vitrolife, Sweden) that was prewarmed to 37 ˚C. The maximum number of flushes was six. OCCCs were denuded after at least two hours of incubation. Following maturation assessment, all metaphase-II (M-II) oocytes were fertilized by standard intracytoplasmic sperm injection (ICSI). Fertilization was assessed 16-17 hours after ICSI, and the presence of two pronuclei represented normal fertilization. Embryos were cultured for 3-5 days, depending on the primary physician's preference. Figure 1 shows the flowchart of the inclusion and exclusion of patients from the study.

The Koç University Local Research Ethics Committee approved the study (approval number: 2020.181.IRB1.049). Informed consent was obtained.

Statistical analysis

The Kolmogorov-Smirnov test was used to check for normality of distribution. All continuous variables displayed a normal distribution. Continuous variables are represented as mean ± standard deviation while categorical variables are described as frequency with rate. The Student's t-test for normally distributed continuous data and chi-square or Fisher's exact tests for categorical data were used for statistical comparison, as appropriate.

The primary endpoint was oocyte retrieval rate in the initial aspirate versus subsequent flushing episodes. The secondary endpoints were oocyte maturity, fertilization and embryo

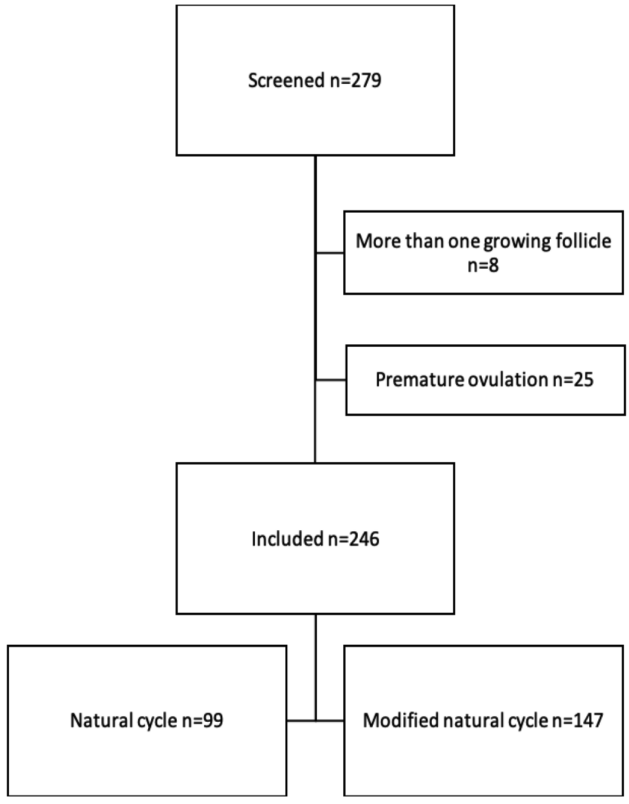

Figure 1. Flowchart of the study population

cleavage rates. Correlation and logistic regression analyses were used to assess the factors related to oocyte retrieval. Several literature-derived and biologically plausible confounders were identified including maternal age, BMI, natural or modified natural cycle, peak E_2 level, and diameter of the follicle at the time of triggering ovulation. All p-values were two-sided and $p<0.05$ was considered significant. Statistical analyses were carried out using the SPSS, version 24.0 (IBM, Chicago, IL, USA).

Results

The study group included 246 OPU procedures in 226 women, for 99 natural cycles and 147 modified natural IVF cycles. Baseline characteristics of all cycles and their outcomes are reported in Table 1. Seven women had multiple treatment attempts. The procedure was successful in 187 (76%) cycles, from which 160 M-II oocytes were retrieved (including five that were developed in vitro from M-I oocytes). The fertilization rate was 53.1% (85/160). On the third day of in vitro culture, these 85 zygotes developed into 23 (27.1%) grade 1 and 55 (64.7%) grade 2 embryos, whereas seven (8.2%) showed cleavage arrest.

Table 2 shows the number of oocytes, M-II oocytes, zygotes and cleaved embryos generated from the oocytes collected from the initial aspirate and subsequent flushing episodes. The initial aspirate contained approximately half (54%) of the total oocyte yield (101/187). The first, second, third and fourth flushes provided 46 (24.5%), 19 (10%), 14 (7.5%) and 7 (4%) oocytes, respectively. No oocytes were recovered thereafter.

Table 1. Characteristics of all cycles and their outcomes

Variable	All
Number	246
Female age (years)	40.1±4.6 (27-49)
Body mass index (kg/m^2)	27.5±3.9 (18.7-43)
Number of previously failed cycles	2.3±1.6 (1-8)
Follicle diameter on hCG day (mm)	17.9±0.9 (16.5-19.5)
Peak E_2 level (mIU/L)	245.2±56.8 (139-413)
*Values are represented as number or mean ± standard deviation (range)	

Figure 2 depicts cumulative percentages of the oocytes retrieved. The odds of retrieving an oocyte were 0.07 [95% confidence interval (CI): 0.05-0.11], if no flushing was performed (p=0.0001).

Among a priori selected confounders, the follicle diameter was positively correlated with the chance of retrieving an oocyte (r=0.185, p=0.040). None of the other factors were related with success in oocyte retrieval (female age: r=-0.030, p=0.635; BMI: r=0.043, p=0.503; peak E_2 level: r=-0.099, p=0.126; natural vs modified natural cycle p=0.595). The lowest E_2 level in a cycle with an M-II oocyte retrieval was 139 pg/mL.

Oocyte maturity was gradually decreased in subsequent flushing episodes, but the difference was not statistically significant (p=0.577). Fertilization rates of M-II oocytes obtained from the initial aspirate and one to three episodes of flushing were comparable (p=0.971). None of the five oocytes obtained from the fourth flush was fertilized.

Cleavage rate of embryos derived from oocytes retrieved from the initial aspirate and one to two episodes of flushing, however, was significantly higher compared to those of embryos derived from the oocytes obtained from the third flushing episode (50%, 3/6, p=0.006).

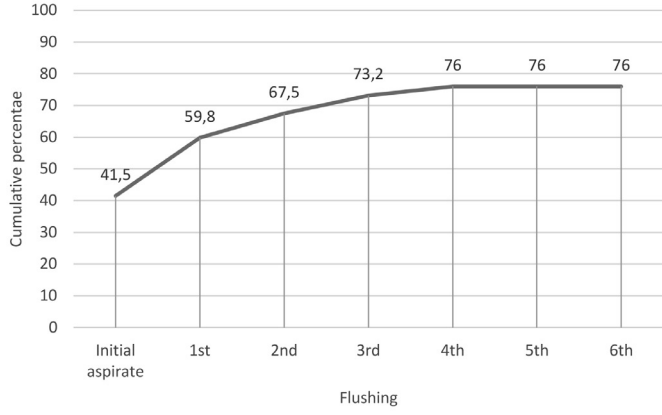

Figure 2. Cumulative percentage of oocyte recovery in 246 procedures

Table 2. Oocyte yield, M-II oocytes, zygotes and cleaved embryos generated from the oocytes collected from the initial aspirate and subsequent flushing episodes

Flushing episode	Oocyte yield	M-II oocyte	Fertilization	Cleavage
Initial aspirate	101 (54)	90 (89.1)	50 (55.5)	47 (94)
1st	46 (24.5)	39 (84.8)	20 (51.3)	20 (100)
2nd	19 (10)	15 (78.9)	9 (60)	8 (88.9)
3rd	14 (7.5)	11 (78.6)	6 (54.5)	3 (50)
4th	7 (4)	5 (71.4)	0	0
Total	187	160 (85.6)	85 (53.1)	78 (91.8)
All data are shown as n (%), M-II: Metaphase-II				

Discussion

This study has shown that follicular flushing increased oocyte recovery rate in mono-follicular IVF cycles. However, no oocytes were retrieved after four flushing episodes. Oocytes obtained from the third flush onward either failed to be fertilized or developed into poor quality embryos.

The benefit of routine flushing in OPU is controversial (3,4). Published reports have concentrated mainly on data derived from multi-follicular growth in stimulated cycles. The latest Cochrane meta-analysis, including 10 randomized controlled trials in 928 women, reported no difference in oocyte yield between direct aspiration versus follicular flushing of multiple follicles (5). Observational studies suggest a potential benefit in cycles with only a few growing follicles (3,6,7). However, data on natural IVF cycles are very limited. Our study showed a clear benefit from flushing in natural and modified natural cycles, as flushing increased the oocyte retrieval rate from 41.1% (101/246) to 76% (187/246). Similarly, Mendez Lozano et al. (8) showed an increase in the oocyte yield from 46.8% to 84.6% in minimally stimulated cycles and von Wolff et al. (9) reported an increase from 44.5% to 80.5% in mono-follicular cycles. A recent randomized trial showed significant increase in the mature oocyte retrieval rate by flushing (77.1% versus 59.3%) (10). Compared to previous reports, the rate of mature oocyte retrieval was lower in our study (65%, 160/246). This might stem from the differences in patient characteristics, as the study groups were much younger in these three earlier studies as the mean female age was 33.5 (20-37), 37.0 (28-45) and 35.0 (18-42), respectively (8-10) compared to 40.1±4.6 years in our population.

Despite the suggested benefit of flushing in minimally stimulated or natural IVF cycles, there is no consensus on the optimal number of flushing attempts. When the initial aspirate does not contain the oocyte, it is likely that the very first flushing would drive the oocyte that remains in the dead space within the lumen of the needle or connecting system into the collecting tube. In a prospective study on stimulated IVF cycles, 40% of the oocytes were obtained in the primary aspirate and 41.3% in the dead space of the collecting system (11). We observed that the last flush that yielded an M-II oocyte was the fourth and an oocyte with fertilization capacity was the third. No oocytes were retrieved after the fourth flushing episode and these findings are comparable with previously published reports. Mendez Lozano et al. (8) harvested 55.5% of oocytes in the direct aspirate, and 44.5% from follicular flushing (80.3% in the first, 10.7% in the second, 5.8% in the third and 2.9% in the fourth flushing). Bagtharia and Haloob (12) reported that direct aspiration provided 40% of the oocytes and the rate was increased to 97% after two to four flushes. von Wolff et al. (9) retrieved 44.5% of oocytes in the primary aspirate, 20.7% in the first, 10.4% in the second and 4.3% in the third flush. Xiao et al. (13) was able to collect an oocyte from the ninth flushing episode but suggested that a reasonable maximum number of flushes was four. Kohl Schwartz et al. (10) reported that the majority of mature oocytes were retrieved in three flushing episodes.

Another concern related with oocytes obtained with flushing is their quality. A prospective study of 300 embryos generated from oocytes retrieved either in initial aspirate or flushing episodes showed that viability, fertilization capacity and cleavage rates were lower in oocytes harvested through flushing (11). During flushing the increase in intra-follicular pressure, longer procedure time, and change in paracrine milieu due to dilution may cause damage to the oocyte, either fracturing the zona or stripping the cumulus mass (4,14). In contrast, Kohl Schwartz et al. (10) showed no association between the number of flushes and quality of embryos [odds ratio (OR): 1.39; 95% CI: 0.93-2.11]. We observed that the last flushing episode that yielded an M-II oocyte was the fourth and for an oocyte with fertilization capacity this was the third. However, fertilization rates in oocytes obtained from the first three flushing episodes were comparable and cleavage rates in embryos generated from the oocytes retrieved in the first two flushing episodes were similar.

Study limitation

Our study has limitations due to its retrospective data collection design. As the study was based on a heterogenous group of poor responder women, the results cannot be generalized to women with good ovarian reserve undergoing natural cycle IVF.

Conclusion

Flushing confers a benefit for oocyte recovery rates in mono-follicular IVF cycles in poor responder women. However, more than three attempts at flushing were not associated with good outcome.

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Ethics Committee Approval: *The study was approved by the Ethical Committee of the Koç University Faculty of Medicine (approval number: 2020.181.IRB1.049).*

Informed Consent: *It was obtained.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: S.E., B.B., B.U., K.Y.; Concept: K.Y.; Design: K.Y., S.E.; Data Collection or Processing: S.E., B.B., K.Y.; Analysis or Interpretation: K.Y.; Literature Search: S.E., K.Y.; Writing: K.Y.*

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Evaluation of peripheral nodal recurrence in patients with endometrial cancer

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Abstract

Objective: To evaluate the clinico-pathological patient features, prognostic factors, treatment options and outcomes of peripheral nodal recurrence (PNR) of endometrial cancer (EC).

Material and Methods: The data of nine patients with PNR of EC from two institutions were reviewed. The electronic literature was reviewed from 1972 to May 2018 to identify articles about PNR in EC. Finally, 42 cases were evaluated.

Results: Nineteen (45.2%) patients were initially diagnosed with either stage I or II disease, whereas 20 (47.7%) patients had stage III or IV disease while the stages were not reported in three (7.1%). PNR developed as the first recurrence in 40 (95.2%) patients and as the second recurrence in 2 (4.8%) patients. Isolated PNR appeared in 35 (83.3%). Seven (16.7%) had PNR coexisting with multiple other sites of tumoral involvement. In the entire cohort, the 5-year and 10-year post-recurrence survival (PRS) were both 78%. Only the presence of distant hematogenous metastasis concurrent with PNR was significantly related to poor PRS ($p=0.005$). Among patients with isolated PNR, those who had surgery had 30% greater 5-year PRS than those treated without surgery, but this difference was not significant (80% vs 50%; $p>0.05$).

Conclusion: A concurrent distant hematogenous metastasis was the only factor related to poor survival. A wide range of therapies exists for PNR but none of the therapies appear to be more advantageous than another. However, surgery as a component of treatment can render a survival advantage for patients who have isolated PNR. (J Turk Ger Gynecol Assoc 2022; 23: 38-50)

Keywords: Endometrial cancer, lymphatic failure, peripheral nodal recurrence, survival, treatment

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Introduction

Endometrial cancer (EC) is the most common gynecological malignancy (1). Although EC has a high disease-free survival rate, its recurrence rate is 13-16% (2,3). EC usually recurs locally in the pelvis or vaginal cuff (4). The lymphatic failure in EC appears mostly in specific retroperitoneal lymph nodes, such as the pelvic and para-aortic nodes (3,5). Therefore, many studies have focused on the prognostic factors and treatment options of these frequently encountered recurrence sites (5-7). Various atypical recurrence sites have been reported (8). Peripheral nodal recurrence (PNR) is one of the rare failure patterns of EC. Due to its infrequency, it is important to detect patients who are at high risk for peripheral lymphatic failure. Treatment options range from local surgical excision to pelvic exenteration, chemotherapy, radiotherapy and palliative therapy (9-11). Furthermore, the limited information on PNR in EC is based solely on cases from the literature. Therefore, PNR treatment options in EC remain unclear.

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In the current study, a case series of PNR from EC is presented. The aim of this study was to evaluate the clinico-pathological patient features, prognostic factors, treatment choices, and outcomes of PNR in EC.

Material and Methods

Data of 1,345 patients with epithelial EC who underwent at least a hysterectomy and bilateral salpingo-oophorectomy in our gynecological-oncology clinic between January 1993 and May 2013 were evaluated. These cases were assessed for the presence of PNR, which was defined as the presence of involved lymph nodes outside the abdominal cavity (except for the mediastinal lymph nodes) in cases with at least a one-month disease-free interval (DFI) following complete response to treatment before PNR. Patients who had a sarcomatous component identified in their histopathological examination or whose peripheral nodal involvement appeared without at least a one-month DFI were excluded. Recurrence developed in 162 of 1,345 cases with epithelial EC. The rate of PNR was 4.9% (8/162) among patients who developed all types of recurrences from epithelial EC. These eight patients from the first institution were added to the study group. One patient from the second participating institution who had PNR was also included (12). Thus, a study group was formed with a total of nine patients from two institutions. The University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Training and Research Hospital Institutional Committee has approved the study protocol (approval number: 47502, date: 25.06.2018). All patients signed an informed consent that allows the institution to use their clinical data.

Literature review

A systematic review of the medical literature was conducted to identify articles about PNR after initial treatment of EC. The electronic literature search was reviewed from 1972 to May 2018 using PubMed/MEDLINE for English language abstracts. The search included the following medical subject headings or keywords: "distant" or "peripheral" or "unusual" or "supraclavicular" or "inguinal" or "neck" or "axillar" or "jugular" lymph node recurrence of EC. After the completion of the search, 29 articles were found. Subsequently, 17 articles were excluded from the study for reasons that are presented in detail in the research chart (Figure 1). In four of the excluded articles, only the locations of the distant lymph nodes were detailed and the distribution of those were: cervical and

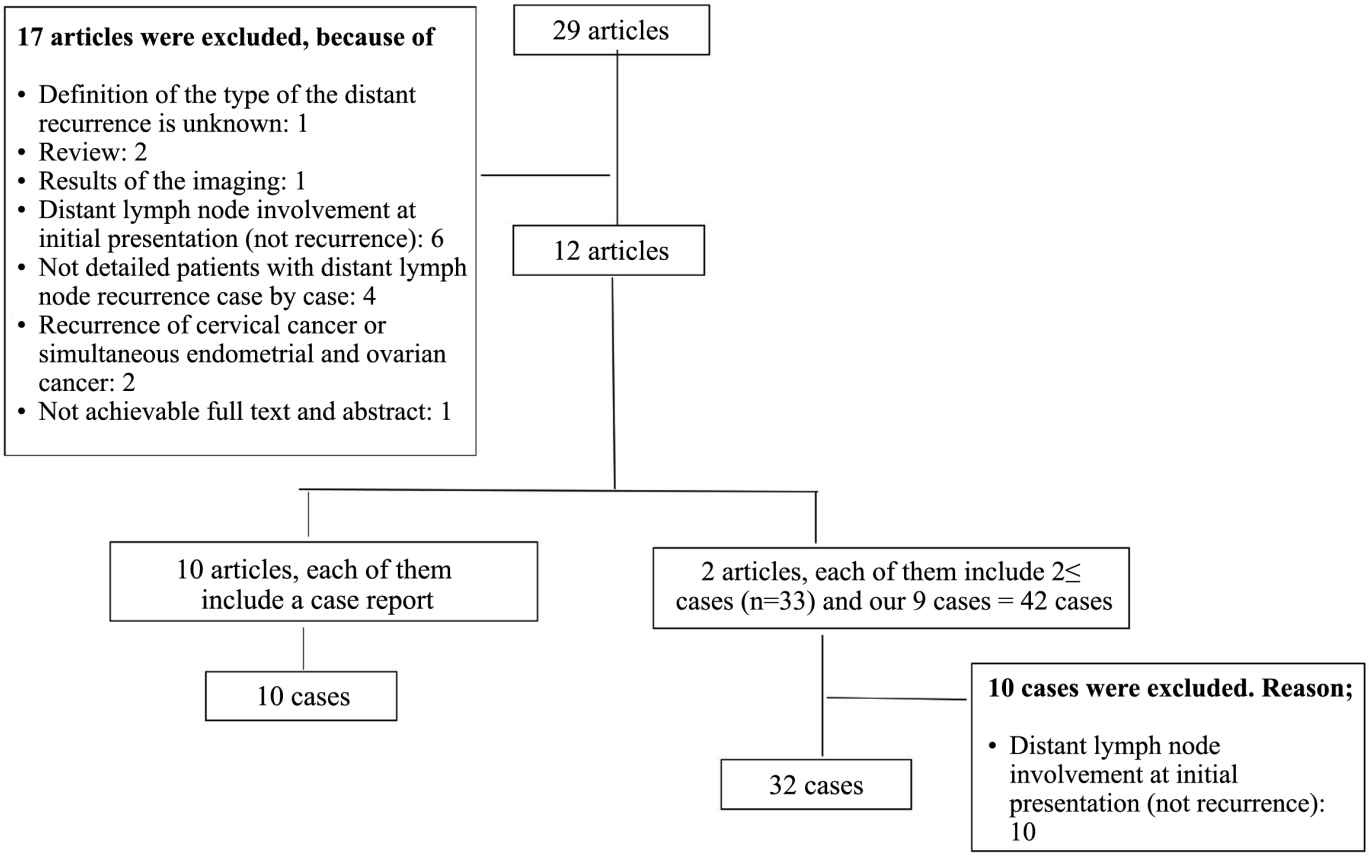

Figure 1. Chart showing details of the literature review

supraclavicular nodes, 5 cases (13); inguinal nodes, 5 cases (13-15); cervical nodes, 5 cases (14); supraclavicular nodes, 2 cases (16); subclavian nodes, 2 cases (14); and axillary lymph nodes, 1 case (16). Therefore, only the frequency of involved nodes for these cases from the four articles was included in the analysis. Cases (n=43) from the remaining 12 articles were evaluated comprehensively. Ten of the eleven cases with peripheral nodal involvement, reported in one article (17) were excluded because they had peripheral nodal involvement at initial presentation (not at recurrence). The follow-up time and end status of a case that had been previously published about PNR of EC was updated (12). Finally, we evaluated a total of 42 cases, including our case series of nine patients.

Data evaluation

Disease recurrence involving the peripheral lymph nodes alone was defined as isolated PNR. Recurrence, which developed in any other location in conjunction with peripheral lymph nodes was defined as PNR with multiple involved sites. Patients were staged according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) criteria (18). Therefore, stages of patients were updated for articles that were published before 2009, if the histopathological findings were available. Tumor size was defined as the largest tumor diameter for a recurrent tumor. Tumors with undifferentiated, clear cell and serous histology were accepted as grade 3 disease. DFI was described as the time period from initial treatment to PNR for patients with the first recurrence and from treatment before PNR to appearance of PNR for patients who had a secondary recurrence. The period from PNR to last patient visit or patient death was defined as post-recurrence survival (PRS). The follow-up time was defined as the interval between initial treatment to death or the last contact with the patient. Involved cervical lymph nodes included PNR that was described as neck, jugular, or cervical in articles from the medical literature. Subclavian lymph node involvement was classified as supraclavicular lymph node involvement.

Patients with suspected PNR were evaluated by clinical examination and radiological imaging methods. Subsequently, the diagnosis of PNR was made based on these findings. Radiological imaging was evaluated by a radiologist. Suspicious peripheral lymph nodes were biopsied. Management of PNR was directed by the institutional tumor board.

Patients who had a complete clinical response after treatment for recurrence were followed-up at three-month intervals for the first two years, at six-month intervals for the next three years, and annually thereafter. Pelvic examination, complete blood count, blood chemistry and abdominopelvic ultrasonography were performed as follow-up monitoring. Chest X-ray was performed yearly unless clinical suspicion indicated otherwise. Abdominal and/or thoracic computed tomography were used when required. Although not routinely used, CA-125 levels were utilized for follow-up.

Statistical analysis

SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for continuous variables and number/percentage for categorical variables. The Kaplan-Meier method was used for the assessment of survival outcomes. Multivariate analysis was performed using a Cox proportional hazards model. All variables with a $p<0.25$ in univariate analysis were included in the multivariate analysis. Survival curves were compared using the log-rank test. A p-value less than 0.05 were considered to be statistically significant.

Results

The median (range) age of the study group was 60 (45-75) years. The histological types were endometrioid adenocarcinoma in 13 (31%), clear cell adenocarcinoma in 3 (7.1%), and mixed cell adenocarcinoma in 1 (2.4%) patient. Mixed cell adenocarcinoma was composed of grade 3 endometrioid adenocarcinoma with 25% mucinous differentiation and 15% clear cell adenocarcinoma. The type of adenocarcinoma was not specified in 22 patients. The differentiation of endometrioid adenocarcinoma was FIGO grade 1 in 7 patients, grade 2 in 3 patients, and grade 3 in 3 patients. In 22 patients, the grade was classified according to the 1988 Broder's classification (Table 1) (19). Distribution of the 2009 FIGO stages was as follows; stage 1, 17 (40.5%) patients; stage 3, 15 patients (35.8%); and stage 4, 5 patients (11.9%). The stages of the two patients (4.8%) with stage 2 disease could not be updated according to the 2009 FIGO criteria because of the absence of information on the type of cervical involvement. The stage was unknown in three patients. Three patients had a history of unopposed estrogen exposure (20) breast cancer (21), and rectal cancer (11), respectively. The clinico-pathological findings of the entire cohort are shown in Table 1, 2.

PNR developed as the first recurrence in 40 (95.2%) patients, while in 2 (4.8%) patients it appeared as the second recurrence. The median DFI was 15 months, ranging between 2 and 276 months. The sites of PNR reported in the four excluded articles were: inguinal lymph nodes in 26 (41.9%); supraclavicular lymph nodes in 22 (35.5%); cervical lymph nodes in 15 (24.2%); and axillary lymph nodes in 5 (8.1%). The median (range) diameter of the recurrent tumor was 3.75 (2-10) cm. Isolated PNR occurred in 35 (83.3%) patients. Seven (16.7%) had PNR with multiple involved sites. Other sites associated with PNR were the vagina including the peri-urethral area (n=1); pelvis

Table 1. Features related to the initial diagnosis of endometrium cancer in the entire cohort: systematic review of the literature

	Case no	A.	Tm type	Stage	Grade (G)	MI	Cx. Inv.	LVSI	Adx. Inv.	Initial treatment	Adjuvant therapy	Disease-free interval (m)	
Aalders et al. (17)	1	46	AC	IVB (inguinal node metastasis)	-	-	-	-	-	Primary RT (pelvic megavoltage) + progestagens (hydroxyl-progesterone caproate)	-	60	
Foote et al. (19)	1	63[b]	AC: 21p UK: 1p	I	Broder's[d]: G1: 2p G2: 9p G3: 7p G4: 3p UK: 1p	-	Absent	-	Absent	Hysterectomy	None	4	Median: 16 m (range, 3 m-10 years)
	2			II[c]		-	Present	-	Absent	Hysterectomy	RT (pelvic)	4	
	3			III		-	-	-	-	Hysterectomy	RT (abdominal)	13	
	4			III		-	-	-	-	Hysterectomy	RT (abdominal)	10	
	5			III		-	-	-	-	Hysterectomy	RT (abdominal)	36	
	6			III		-	-	-	-	Hysterectomy	RT (abdominal)	17	
	16p[a]			I: 9p II: 1p[c] III: 3p IV: 1p (omental met.) UK: 2p		-	-	-	-	Hysterectomy ± BSO: 15p Primary RT: 1p	None: 3p RT (pelvic): 8p RT (abdominal): 2p RT (intrauterine radium): 1p Hormonal therapy: 1p		
Carr et al. (20)	1	52	EAC	IA	G1	<1/2	Absent	UR	Absent	TH + BSO	None	12	
Wu et al. (31)	1	55	-	-	-	-	-	-	-	TH + BSO + pelvic LND	RT (VBT)	-	
Bilici et al. (32)	1	67	EAC	IIIC	G3	≥1/2	Absent	Present	Absent	TH + BSO + pelvic LND	RT (50.4 Gy pelvic + VBT)	15	
Alameda et al. (21)	1	72	EAC	IIIB	G1	Present	Absent	-	-	TH + BSO	None	8	
Ortaç and Taşkın (12)	1	45	EAC	IA	G2	<1/2	Absent	Absent	Absent	TH + BSO + Paraaortic-pelvic LND + partial omentectomy	None	7	
Kojima et al. (11)	1	74	-	IIIC	-	-	-	-		TH + BSO + pelvic LND	CT → after 12m →PA nodal rec.→ PA lymphadenectomy + CT	36	
Seagle et al. (33)	1	67	EAC	IB	G1	-	Absent	-	Absent	TH + BSO + pelvic LND	RT (VBT)	14	
Margolis et al. (9)	1	48	EAC	IIIC2	G3	≥1/2	Absent	Present	Absent	TH + BSO + paraaortic-pelvic LND	CT (carboplatin -paclitaxel) + RT (4500 cGy pelvic and 5040 cGy)	17	

Table 1. Continued

	Case no	A.	Tm type	Stage	Grade (G)	MI	Cx. Inv.	LVSI	Adx. Inv.	Initial treatment	Adjuvant therapy	Disease-free interval (m)
Akbar et al. (10)	1	65	EAC	IA	G3	<1/2	Absent	Present	Absent	TH + BSO	None	16
Yordanov et al. (34)	1	65	EAC	IA	G2	<1/2	Absent	Absent		TH + BSO	RT (54 Gy pelvic)	276
Present study 2018	1	66	Clear cell AC	IA	-	<1/2	Absent	-	Absent	TH + BSO + paraaortic-pelvic LND	CT (cisplatin)	45
	2	60	EAC	IIIC2	G1	≥1/2	Absent	-	Absent	TH + BSO + paraaortic-pelvic LND	RT (4500 cGy pelvic and 5040 paraaortic)	38
	3	60	Clear cell AC	IVB (L. Supraklavicular LN)	-	≥1/2	Present	-	Absent	TH + BSO + paraaortic-pelvic LND	CT (cisplatin + adriamisin)	5
	4	58	EAC	IVB (umblicus met.)	G1	≥1/2	Absent	Present	Absent	TH + BSO + paraaortic-pelvic LND	CT (carboplatin + paclitaxel)	84
	5	50	EAC	IA	G1	<1/2	Absent	Present	Absent	TH + BSO + paraaortic-pelvic LND	None	30
	6	61	Clear cell AC	IIIC2	-	Confined to end.	Present	-	Absent	TH + BSO + paraaortic-pelvic LND	CT (3 cycles carboplatin + paclitaxel; because of the side effects she refused the therapy)	3
	7	60	EAC	IIIC2	G2	≥1/2	Absent	Present	Absent	TH + BSO + paraaortic-pelvic LND	RT	10
	8	75	EAC	IIIC2	G1	≥1/2	Absent	Absent	Absent	TH + BSO + paraaortic-pelvic LND	CT (after 1 cycle carboplatin + paclitaxel, she refused the therapy)	32
	9	59	Mixt AC (endometrioid + Mucinous + clear cell)	IIIA	G3	≥1/2	Present	Present	Present	TH + USO (previous USO history)	CT (6 cycles carboplatin + paclitaxel) → after 8m → vaginal cuff + left internal iliac LN rec → CT (paclitaxel + carboplatin)	2

A: Age (years), cx.: Cervical, adx: Adnexal, inv.: Involvement, LN: Lymph node, Tm: Tumor, p.: Patient(s), UK: Unknown, AC: Adenocarcinoma, EAC: Endometrioid adenocarcinoma, MI: Myometrial invasion, end: Endometrium, LVSI: Lympho-vascular space invasion, RT: Radiotherapy, TH: Total hysterectomy, USO: Unilateral salpingo-oophorectomy, BSO: Bilateral salpingo-oophorectomy, LND: Lymphadenectomy, CT: Chemotherapy, VBT: Vaginal brachytherapy, FIGO: International Federation of Gynecology and Obstetrics, [a]: The remaining 16 patients, [b]: Median age of 22 patients, [c]: Stage II could not be updated according to 2009 because of the absence of the involvement type of cervix, [d]: Grade classification type (in 1988)

Table 2. Features of the entire cohort

Findings		n	%
Stage	I	17	40.5
	IA	6	14.3
	IB	1	2.4
	US stage I	10	23.8
	II[a]	2	4.8
	III	15	35.8
	IIIA	1	2.4
	IIIB	1	2.4
	IIIC	7	16.7
	IIIC2	5	11.9
	US	2	4.8
	US stage III	6	14.3
	IV	5	11.9
	IVB	3	7.1
	US stage IV	2	4.8
	UR	3	7.1
Histologic type	Endometrioid	13	31.0
	Grade 1	7	16.7
	Grade 2	3	7.1
	Grade 3	3	7.1
	Clear cell AC	3	7.1
	AC (not specified)	22	52.4
	Mixed cell AC (grade 3 endometrioid + mucinous + clear cell)	1	2.4
	UR	3	7.1
Myometrial invasion[b]	Confined to endometrium	1	1.6
	Presence of myometrial invasion	16	25.8
	Invasion <1/2	6	9.7
	Invasion ≥1/2	9	14.5
	US	1	1.6
	UR	45	72.6
Site of recurrent peripheral lymph node[b]	Axillar	4	6.4
	Right	1	1.6
	Left	1	1.6
	US	2	3.2
	Inguinal	26	41.9
	Right	9	14.5
	Left	10	16.1
	US	7	11.3
	Supraclavicular	16	25.9
	Right	8	12.9
	Left	4	6.5
	US	4	6.5
	Cervical	10	16.1
	Left	3	4.8
	US	7	11.3
	Cervical + supraclavicular	5	8.1
	Axillar + supraclavicular	1	1.6

Table 2. Continued

Findings		n	%
Involvement pattern	Isolated PNR	35	83.3
	PNR with multiple involved sites	7	16.7
Status of the distant recurrence sites other than PNR	Absent	40	95.2
	Present	2	4.8
Therapy options at recurrence[c]	Radiotherapy + hormone therapy	1	2.4
	Only chemotherapy	5	11.9
	Chemotherapy + radiotherapy	1	2.4
	Chemotherapy + hormone therapy	1	2.4
	Only surgery	2	4.8
	Surgery with adjuvant therapy	13	31
	Surgery + radiotherapy	6	14.3
	Surgery + chemotherapy	5	11.9
	Surgery + chemo-radiotherapy	1	2.4
	Surgery + chemotherapy + radiotherapy	1	2.4
	Surgery + hormone therapy	1	2.4
	UR	2	4.7
End status	AWOD	16	38.1
	DOD	18	42.9
	AWD	2	4.8
	LFU	3	7.1
	UR	3	7.1

PNR: Peripheral nodal recurrence; UR: Unreported; AWOD: Alive without disease; AWD: Alive with disease; LFU: Lost to follow-up; US: Unspecified, DOD: Dead of disease, [a]: Could not updated according to FIGO 2009 because of the absence of the involvement type of cervix, [b]: The distribution of the location analyzed among the 62 patients, [c]: 16 patients from report of the Foote et al. (19) were excluded because the therapy type was not given case by case

(n=1); retroperitoneal lymph nodes (n=2); and retroperitoneal lymph nodes together with involvement of the central pelvis (n=1). In addition, two patients had distant organ metastasis (liver parenchyma with or without the tail of the pancreas) concurrent with PNR. Details of the features of recurrent disease are given in Table 2, 3.

The rate of initial nodal involvement was higher in patients with inguinal PNR than patients with other sites of PNR [70% (7/10) vs 18.2% (2/11), p=0.03]. The frequency of the presence of cervical invasion was higher in patients with PNR localized in the supraclavicular nodes than in patients with PNR sites besides the supraclavicular nodes [100% (2/2) vs 12.5 (2/16); p=0.039].

In 16 (39.2%) patients, surgery was performed for the treatment of PNR. Seven (19.1%) had non-surgical treatment, including chemotherapy (n=5), chemotherapy with radiotherapy (n=1), hormonal therapy with radiotherapy (n=1) and hormonal

Table 3. Post-recurrence features of the entire group: systematic review of the literature

	Case no.	Which rec.	Type of involved peripheral LN[a]	Sizeof tm[a] (cm)	No. of the OIS[a]	Location of the OIS[a]	Presence of the other distant sites	Therapy	Postrec. situations	End status	FU time	
Aalders et al. (17)	1	First	Axillary	-	Isolated	-	No	RT + HT (progestagens)	-	AWOD	120	
Foote et al. (19)	1	First	R. inguinal	4	Isolated	-	No	S + CT (5-FU)	-	AWOD	205	Median: 34.5 m (7 m-17 years)
	2	First	R. supra-clavicular	2	Isolated	-	No	S + HT (progestagens)	-	AWOD	27	
	3	First	R. axillary	4.5	Isolated	-	No	S + RT (5000 Gy≤)	-	AWOD	45	
	4	First	R. inguinal	3	Isolated	-	No	S + RT (5000 Gy≤)	-	AWOD	31	
	5	First	R. inguinal	4	Isolated	-	No	S + RT (5000 Gy≤)	-	AWOD	59	
	6	First	L. supra-clavicular	3	Isolated	-	No	S + RT (5000 Gy≤)	-	AWOD	53	
	16pb)	All first	R. inguinal: 3p L. inguinal: 4p R. supra-clavicular: 7p L. supra-clavicular: 1p L. axillary + supra-clavicular: 1p	<4: 8p 4≤: 7p UK: 2p	Isolated	-	No	S + RT: 6 S + RT + HT: 2 RT: 1 S + CT: 2 (5-FU: 1; doxorubicin: 1) S + CT + HT: 1 S + HT: 5 (therapy distribution was given for 17 nodes of 16p)	16p had re-recurrence (postrec. DFI: 6 m (1-33 m)	DOD: 15p AWD: 1p	-	
Carr et al. (20)	1	First	L. inguinal	9*7	Multiple	LN (celiac and porta hepatis)	No	CT (cyclophosphamide +carboplatin +HT (megestrol acetate)	-	AWOD	12	
Wu et al. (31)	1	First	Inguinal	UR	Multiple	Bulky central rec. and pelvic-paraaortic nodes	No	S + whole pelvic chemo-RT (with concurrent cisplatin)	Mediastinal and neck nodal involvement appeared (during treatment) → carboplatin + paclitaxel → Neck node RT and epirubicin → 10 m later → central re-rec. → pelvic exenteration → for 5 years disease free	AWOD	At least 70	

Table 3. Continued

	Case no.	Which rec.	Type of involved peripheral LN[a]	Sizeof tm[a] (cm)	No. of the OIS[a]	Location of the OIS[a]	Presence of the other distant sites	Therapy	Postrec. situations	End status	FU time
Bilici et al. (32)	1	First	L. -anterior cervical	2*2	Isolated	-	No	CT (doxorubicin + cyclophosphamide + cisplatin)	-	AWOD	21
Alameda et al. (21)	1	First	L. axillary	UR	Isolated	-	No	UR	-	UR	UR
Ortaç and Taşkın (12)	1	First	R. inguinal	4*5	Isolated	-	No	S + RT	Re-recurrence occurred	DOD	43
Kojima et al. (11)	1	Second	L. supra-clavicular	UR	Isolated	-	No	S	-	AWOD	48
Seagle et al. (33)	1	First	L. inguinal	10*7.5	Isolated	-	No	CT (carboplatin + paclitaxel) + pelvic RT + inguinal LN boost RT (25 Gy)	-	UR	UR
Margolis et al. (9)	1	First	L. inguinal	1.8*2.6	Multiple	Vagina including peri-urethral area	No	S (anterior pelvic exenteration) + CT (carboplatin + gemcitabine)	-	AWOD	120
Akbar et al. (10)	1	First	L. inguinal	2.4*2.6	Multiple	LN (right external and left paraaortic)	No	S + pelvic-paraaortic-bilateral inguinal RT and inguinal LN boost RT (with concurrent cisplatin) + VBT + CT (carboplatin + docetaxel)	-	AWOD	29
Yordanov et al. (34)	1	First	L. inguinal	4*5	Isolated	-	No	S + RT (30 Gy)	-	AWOD	294
Presented study 2018	1	First	Inferior jugular	UA	Multiple	Liver parenchyma, tail of the pancreas	Yes	UA	-	LFU	45
	2	First	L. jugular	4.5*3.5	Isolated	-	No	CT (carboplatin + adriamycin)	2 Cycles CT → progression (in neck involvement and addition of axillar lymph node involvement) → instability due to the other vital systems → palliative therapy	DOD	45

Table 3. Continued

	Case no.	Which rec.	Type of involved peripheral LN[a]	Sizeof tm[a] (cm)	No. of the OIS[a]	Location of the OIS[a]	Presence of the other distant sites	Therapy	Postrec. situations	End status	FU time
Presented study 2018	3	First	L. supraclavicular	3*3	Multiple	Pelvic mass	No	CT (paclitaxel) → Stabile disease → progestagens (megestrol acetate)	-	AWD	19
	4	First	R. inguinal	3*2	Isolated	-	No	S (inguinal lymph node excision) CT (6 cycles, liposomal doxorubicin + cisplatin)	36 m later → Re-recurrence on psoas muscle → S + RT → 5 m later → R. inguinal rec. → RT	AWOD	132
	5	First	R. inguinal	9*8	Isolated	-	No	S + CT	47 m later → Abdominal re-recurrence:	AWD	88
	6	First	L. Inguinal	3*4	Multiple	Liver parenchyma	Yes	S	6 m later → Pelvic and abdominal rec.	DOD	15
	7	First	Cervical	3*3	Isolated	-	No	CT (paclitaxel + cisplatin, 4 cycles)	-	LFU	13
	8	First	L. Jugular	3.5*3	Isolated	-	No	CT (paclitaxel + carboplatin; 5 cycles)	After the 4. cycles, the diameter of tumor reduced to 1 cm according to imaging	LFU	36
	9	Second	L. inguinal	UA	Isolated	-	No	Surgery + CT (cisplatin + adriamisin)	-	AWOD	38

Rec.: Recurrence, LN: Lymph nodes, Tm: Tumor, p.: Patient(s), UK: Unknown, UA: Unavailable, UR: Unreported, DFI: Disease-free interval, FU: Follow-up, AWOD: Alive without disease, AWD: Alive with disease, DOD: Dead of disease, LFU: Lost to follow-up, S: Surgery, RT: Radiotherapy, CT: Chemotherapy, HT: Hormonal therapy, 5-FU: 5-fluorouracil, VBT: Vaginal brachytherapy, No: Number, OIS: Other involved sites, R.: Right, L.: Left, [a]: At recurrence, [b]: The follow-up time and end status updated

therapy with chemotherapy (n=1). The treatment modality was unknown in two patients. The remaining 16 patients could not be grouped based on treatment modality because the type of therapy was not reported for each case so these patients were not included in the survival analysis (19).

The median (range) PRS was 22 (3-201) months. The 5-year and 10-year PRS were both 78%. The median follow-up time was 45 (12-294) months. During follow-up, 18 patients dead of disease. In addition, two patients were alive with disease, 16 patients were alive without disease, three patients were lost to follow-up and the final status of three patients was not reported.

In univariate analysis, the presence of distant hematogenous metastasis, as seen with PNR, was significantly associated with poor PRS (p=0.005). The five-year PRS was 83% for patients who did not have distant hematogenous metastasis during PNR, whereas the patient who had distant hematogenous metastasis with PNR did not survive beyond 5 years (Figure 2). While the five-year PRS of the patients who had PNR with >4 cm diameter was 50%, all of those with ≤4 cm PNR survived passed 5 years (p=0.09). Age, stage, histological type, DFI, the presence of recurrence before PNR, location or side of the recurrence, the diameter of the recurrent tumor, the presence of any other recurrences concurrent with PNR, and treatment types were not significantly associated with PRS. The relationship between clinico-pathological factors and PRS is shown in Table 4. Based on the analysis of the treatment options for isolated PNR (n=18), patients undergoing surgery had a 30% higher 5-year PRS than those who did not undergo surgery. However, this difference was not significant (80% vs 50%; p>0.05).

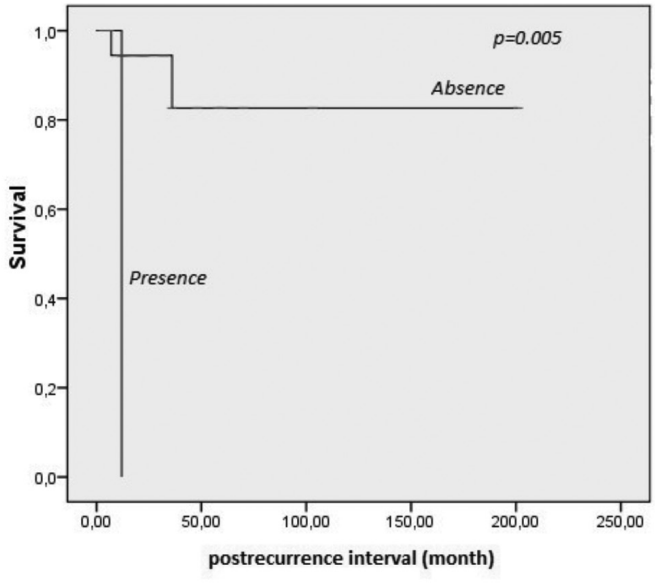

Figure 2. The presence of distant hematogenous metastasis, as seen with peripheral nodal recurrence, was significantly related to poor post-recurrence survival

Variables which were associated with a p<0.25 in univariate analysis were tested in the multivariate analysis. The multivariate analysis model included tumor diameter (>4 cm vs ≤4 cm) and the presence of distant hematogenous metastasis coexisting with PNR (absent vs present). Multivariate analysis revealed that none of the variables was an independent prognostic factor for PRS (Table 5).

Discussion

The present study showed that the most common site of PNR were the inguinal lymph nodes. The major finding of our study was that concomitant hematogenous metastasis with PNR was related to poor PRS. Our study showed that no treatment options for PNR were superior to others.

Peripheral lymphatic failure is extremely rare in EC. The frequency of PNR was 1.92% in all EC cases and 9.3% among recurrent cases with EC (13). In our center, the frequency of PNR was 0.59% and 4.9% within the entire cohort and the group of patients with recurrent EC, respectively.

The most common lymphatic failure sites were the external iliac nodes (22). Kurra et al. (8) reported that the left supraclavicular lymph nodes are the most common distant lymphatic failure sites in EC. In our study, the most common site of PNR was the inguinal lymph nodes. The mechanisms underlying PNR remain unclear. One of the major mechanisms is thought to be the flow of tumoral cells via the thoracic duct (8). Although this explains tumor spread to the supraclavicular area, it cannot account for the inguinal nodal involvement in EC. Carr et al. (20) suggested that unopposed estrogen can cause proliferation of tumor cells in the lymphatic channels of the round ligament. However, only one of the cases with inguinal recurrence had a history of unopposed estrogen based on our literature review. The other hypothesis for isolated PNR is that there is a possibility of missing a metastasis due to the poor value of preoperative imaging in the detection of inguinal micrometastasis, especially for advanced disease (10). There is also a lower rate of detection of micrometastasis on initial evaluation of the retroperitoneal lymph nodes for early stages.

Foote et al. (19) reported that the five-year PRS was 12% for patients with isolated PNR. In our analysis, the five-year PRS was 78%. One of the most likely reasons for the higher survival rate could be the advances in imaging that help in the early detection of recurrence and the high detection rate of metastases in other sites. The factors related to the prognoses of distant recurrences in EC vary (22-26). Only the presence of concomitant distant recurrence with PNR was associated with poor prognosis in PNR, although none of the factors affect the prognosis independently, according to our analysis.

A wide range of options exists for PNR treatment, including local excision, pelvic exenteration, chemotherapy, and

Table 4. The relation between clinico-pathologic factors and post-recurrence survival

		n	5-year PRS (%)	p
Age[a,b] (years)	<60	10	89	0.186
	≥60	6	75	
Stage	1&2	6	67	0.890
	3&4	15	83	
Histologic type[a]	Endometrioid	10	86	0.577
	Non-endometrioid	3	67	
DFI (months)[b]	<15	9	44	0.339
	≥15	12	90	
Presence of the rec. before PNR	Absent (first rec.)	20	77	0.622
	Present (second rec.)	2	100	
Site of recurrence	Inguinal	12	76	0.952
	Others	10	86	
Recurrence site	Right	8	75	0.453
	Left	11	78	
Diameter of the tumor at recurrence[b]	<4 cm	10	100	0.090
	≥4 cm	8	50	
Presence of multiple involved sites during PNR	Isolated PNR	17	77	0.784
	PNR with multiple involved sites	5	80	
Presence of the concomitant distant hematogenous metastasis during PNR	Absent	21	83	**0.005***
	Present	1	None	
Therapy options at recurrence	Surgery vs no surgery			
	Surgery	16	80	0.299
	No surgery	6	67	
	CT absent vs CT present			
	CT absent	10	60	0.525
	CT present	12	88	
	RT absent vs RT present			
	RT absent	13	80	0.584
	RT present	9	75	

PRS: Post-recurrence survival, DFI: Disease-free interval, PNR: Peripheral nodal recurrence, CT: Chemotherapy, RT: Radiotherapy, rec.: Recurrence, *p<0.05 is statistically significant, [a]: Two-year survival, [b]: Median value

Table 5. Multivariate analysis of factors predicting post-recurrence survival after peripheral nodal recurrence

	Hazard ratio (95% CI)	p
Model		
Diameter of the tumor at recurrence (<4 cm vs ≥4 cm)	285164.3 (0.001- …)	0.973
Presence of concomitant distant hematogenous metastasis during PNR (absent vs present)	6.4 (0.405-103.8)	0.187
*P<0.05 is statistically significant, CI: Confidence interval, PNR: Peripheral nodal recurrence		

radiotherapy. Treatment may also include a combination of these therapies and palliative therapy. Unfortunately, there are still no accepted criteria to aid in choosing the type of therapy for PNR. Surgical resection has an important value in isolated distant recurrence of EC, and the probability of achieving complete resection is an important consideration in choosing surgery (24,26-28). However, based on recent knowledge, the necessity of multimodal therapies, especially systemic therapy, cannot be applicable, even for patients with negative margins following complete resection (29). In our study, no specific

treatment had prognostic or survival superiority over any other. Therefore, the management approach in PNR is still at the discretion of the physician and also dependent upon patient preference. However, although not statistically significant, our results indicate that surgery could provide some survival advantage. Therefore, surgical treatment should be kept in the forefront as one component of treatment for isolated PNR. Similar to the interval of onset of other EC recurrences (29-33), 80% of PNR appeared in the first three years. However, PNR can develop as late as 23 years after initial diagnosis (34). Furthermore, a considerable number of patients had stage I disease (40.5%) at initial diagnosis and developed PNR as their first recurrence. Therefore, long-term, close follow-up is critical for early diagnosis.

Study limitation

One of the limitations of the study is its retrospective design. Due to the differences in treatment approaches such as various doses of therapy, chemotherapeutic agents, radiotherapy equipment used, and surgical techniques, distinct conclusions cannot be drawn about outcomes of therapy. Although the other limitation appears to be a small sample size, our study included a relatively large sample of patients with PNR, which results from an extremely rare failure of EC. As far as we know, this is the first and largest study to evaluate factors associated with survival following peripheral nodal failures in EC patients.

Conclusion

Peripheral lymphatic failure was frequently localized in the inguinal lymph nodes. A concurrent distant hematogenous metastasis was the only factor related to poor survival. A wide range of therapies exists but none of the therapies appear more advantageous than any other. However, surgery can provide a survival benefit in patients who have isolated PNR. Further large-scale studies are needed to make definitive conclusions regarding treatment options.

Ethics Committee Approval: *The study was approved by the Ethical Committee of the University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Training and Research Hospital (approval number: 47502, date: 25.06.2018).*

Informed Consent: *All patients signed an informed consent that allows the institution to use their clinical data.*

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This article will be credited by TMA within the scope of CME/CPD.

Efficacy of hyoscine in pain management during hysteroscopy: a systematic review and meta-analysis

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Abstract

We conducted a systematic review and meta-analysis of relevant clinical trials from full-text, scientific journal archives to assess the efficacy of hyoscine for the management of pain during in-office hysteroscopy (OH) procedures. Cochrane CENTRAL, ClinicalTrials.Gov, MEDLINE, PubMed, SCOPUS and the Web of Science were searched for all clinical trials that matched our search criteria. A full assessment of bias was made using the Cochrane Group tool-set. The following outcomes were included: visual analogue scale (VAS) score for postoperative pain, postoperative need for analgesia, and procedure time. In the case of homogeneous data, the analysis was performed using a fixed effects system, and the random effects system was used with heterogeneous data. Inclusion criteria included only randomized clinical trials, and interventions that included patients receiving hyoscine-N-Butyl Bromide during OH, regardless of dose or mode of administration, and compared this with placebo. Three clinical trials were included. The actual mean difference (MD) of the VAS pain score showed no significant difference between hyoscine or placebo [MD: -0.28 (-1.08, 0.52), (p=0.49)]. For postoperative analgesia, the overall MD showed no significant difference between hyoscine or placebo [MD: 0.43 (0.16, 1.14), (p=0.09)]. For procedure time, the combined effect estimate failed to show any significant difference between hyoscine and placebo [MD: -0.66 (-2.77, 1.44) (p=0.54)]. Contrary to previously published data, our meta-analysis using the latest available RCTs fails to show hyoscine as being effective in reducing pain or the need for other forms of anesthesia in OH. (J Turk Ger Gynecol Assoc 2022; 23: 51-7)

Keywords: Office hysteroscopy, hyoscine; office surgery, ERAS protocol, ERAS hysteroscopy

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Introduction

Hysteroscopy is considered the most accurate tool in the diagnosis of disorders of the endometrial cavity (1,2). Office hysteroscopy (OH) carries most of the benefits of hysteroscopy performed under general anesthesia in the operating room, but has many other advantages. Thus, in the opinion of many surgeons, OH represents a cornerstone for both diagnosis and treatment of many gynecological conditions, such as submucosal polyps or leiomyoma (3). OH is also of importance in the diagnosis and management of other pathologies, such as recurrent miscarriage and infertility (4). Prior to the advent

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of hysteroscopy, the management of intrauterine pathology was based largely on blind curettage of the uterus (5). Blind curettage could provide some important information, but dilatation and curettage (D&C) is limited for the recognition of focal lesions, which can result in a higher proportion of false negative results (5). D&C also requires a higher degree of anesthesia to tolerate, usually being performed under general or spinal anesthesia (5).

Conventional hysteroscopy, performed for diagnostic purposes, employs specula and may require dilation of the cervix (6). In recent years, the use of cervical dilators has been widely replaced by the introduction of smaller "mini-hysteroscopes," which limit the need for cervical dilation prior to the procedure (7). Despite these advances, intraoperative pain remains a major problem limiting the use of hysteroscopy. It can be challenging for the hysteroscopist to perform a hysteroscopy without the use of an anesthetic (8). Introducing even a small hysteroscope into the uterine cavity through the cervical canal may produce severe discomfort and pain, especially in sensitive patients (9). The use of sedation, local anesthesia, and cervical ripening agents, such as vaginal misoprostol, have all been utilized in attempts to reduce this pain (10). Hyoscine-n-butyl bromide (HBB) is a peripheral anticholinergic and does not readily cross the blood-brain barrier (11,12). Its mechanism of action is to block the nerve impulses that originate in the parasympathetic ganglia within the abdomen (13). Through blocking the muscarinic receptor, it exerts a spasmolytic action on muscle tissues of the biliary, gastrointestinal and genital organs, with smooth muscles being most affected (13,14). It has been hypothesized that the mechanism of pain reduction by HBB might be the blockage of these impulses, which may prevent uterine spasms (14).

There are few randomized controlled trials (RCTs) investigating the effectiveness of different premedications administered for control of pain during and after OH. A previous meta-analysis failed to find any evidence of the benefit of administration of opioids during OH, when administered orally (15). Another study, this time an RCT, showed that certain anti-inflammatory medications were effective in reducing pain associated with OH, but this was complicated by the addition of a second variable as the study only considered the use of smaller (5 mm) hysteroscopes (16).

Given the scarcity of good evidence, the aim was to conduct a meta-analysis to assess the effect of HBB in women undergoing OH for reducing postoperative pain assessed using the conventional visual analogue pain scale (VAS) score and also the need for postoperative analgesia. It was planned to use the latest available RCTs to produce the highest quality data possible.

Methods

This meta-analysis conformed strictly to the "Preferred Reporting Items for Systematic Reviews and Meta-analyses" (PRISMA) (17) guidelines. In addition, every stage of the study was performed in accordance with the recommendations of the "Cochrane Handbook for Systematic Reviews of Interventions" (18).

Literature search

Six databases were investigated for studies providing evidence about the topic. These were: Web of Science, SCOPUS, Cochrane CENTRAL, ClinicalTrials.Gov, MEDLINE, and PubMed, from inception until January 2021. We followed this search strategy with no restriction on time or languages; [(HBB OR Hyoscine OR Scopolamine OR Buscopan) AND hysteroscopy].

Eligibility criteria

Studies were included according to five criteria: 1) Patient population: patients receiving outpatient hysteroscopy; 2) Intervention: HBB administration regardless of the dose and the mode of administration; 3) Comparator: placebo; 4) Primary outcomes: recorded VAS score during and after OH, as well as usage of postoperative analgesia, while a secondary outcome was the total duration of the procedure (in minutes); and 5) Included study types: only RCTs. Exclusion criteria included: 1) any non-randomized controlled clinical trials; 2) studies that did not report data for the selected outcomes; 3) trials without the full text available; and 4) trials with only a single arm.

Screening process

After results were retrieved from the search, the data was entered into dedicated meta-analysis software (Endnote X8.0.1 Build 1044), where duplicates were removed automatically. The first step was to screen the title and abstract, and this was followed by screening of the entire text. Two different researchers screened each article before final inclusion. Any disagreement was resolved by consensus with a third researcher.

Extraction and analysis of data

Following the completion of screening, data was extracted from the selected studies. The selected data was classified into three categories. The first category was demographic data of the patients, including age, weight, height, body mass index (BMI), number of previous cesarean sections, and history of pelvic pain. The second data category was the indication for the performed hysteroscopy. The final data category included the postoperative VAS score, whether or not postoperative analgesia was required, and the elapsed procedure time in

minutes. In addition, data required for full assessment of risk of bias (ROB), according to Cochrane's ROB tools, was also extracted (19).

Analysis of data

Review Manager Software (version RevMan 5.4.1) was used to perform the analysis using the inverse variance method. The mean difference (MD) and standard deviations were used to express continuous data with a relative 95% confidence interval (CI). Dichotomous outcomes were expressed using percentage and total, relative to a 95% CI. Inconsistency between the studies was assessed by both the I-square test (I2), and the chi-square test to give a p-value. Any outcomes with $I^2 > 50\%$ and $p < 0.1$ were considered to be heterogeneous, while outcomes with $I^2 < 50\%$ and $p > 0.1$ were considered homogeneous, as recommended by the Cochrane Handbook (20). Data that was homogenous was analyzed using a fixed-effects model, while heterogeneous data was analyzed using a random-effects model.

Quality assessment

Quality assessment was performed in accordance with the "Grading of Recommendations, Assessment, Development, and Evaluations" (GRADE) guidelines. The analysis only included RCTs and all other observational evidence was excluded. Cochrane's ROB tool was used to assess ROB for the included RCTs (21). The characteristics assessed by this ROB tool include: 1) proper randomization; 2) proper blinding of the study participants into each group; 3) proper blinding of participants only (single-blinding), blinding of both personnel and participants (double-blinding), or the absence of any blinding; 4) bias attributed to attrition; 5) bias attributed to selection; 6) proper blinding of the outcome assessor (i.e. whether blinded or not); and 7) other biases. The total ROB for these studies was assessed and graded as good.

Results

Summary of included studies

A PRISMA flow diagram of the study literature search is shown in Figure 1. This study included an analysis of 291 patients from three studies (16,22,23). Of these 291, 144 (49.5%) received hyoscine, and 147 (50.5%) were in the placebo group. The mean age of the participant in the treatment group was 38.1±8.7 years, and that of the control group was 39.3±7.8 years. The mean BMI of patients receiving hyoscine was 26.9±6, while that of the control group was 27±5. Table 1 shows a detailed summary of the included participants from each included study. Additionally, Table 2 illustrates the indications for OH.

Results of risk of bias assessment

The ROB analysis indicated an overall low ROB according to Cochrane's tool (24). All studies were judged to be at low ROB from poor randomization. Two of the studies (16,22) reported adequate allocation concealment, and therefore they were considered a low ROB. One study (23) did not report enough data about allocation concealment thus was considered to be an unclear ROB. All of the included studies were double-blinded and so were judged to be free from participant and personnel blinding bias. Two studies (16,22) were judged to be at a low ROB from failing to blind the outcome assessment, except Souza et al. (23) which did not report sufficient details and so was considered an unclear ROB. Again, two studies (16,22) were judged to be at low risk of attrition bias, except Souza et al. (23) which was found to be at high ROB, secondary to a lack of reporting sufficient details about the described outcomes. All of the remaining domains of the Cochrane tool were at a low ROB. A summarized illustration (Figure 2) shows the bias assessment results for the three included studies.

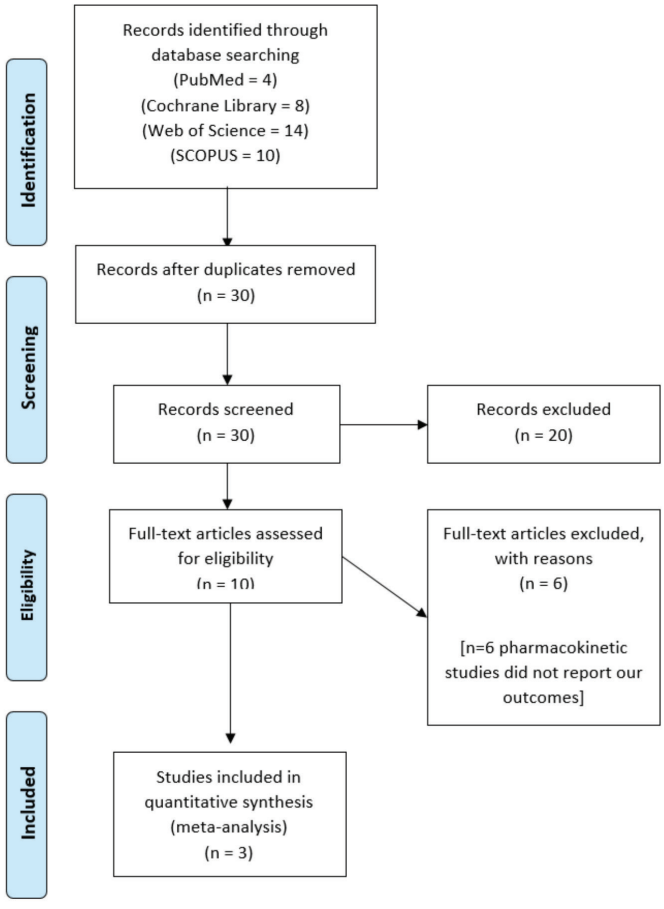

Figure 1. PRISMA flow diagram of the literature search
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analyses

Analysis of all outcomes

1. Postoperative VAS score

All studies (291 participants) reported the postoperative VAS score for pain. Of these, 144 patients were in the hyoscine group, and 147 patients were in the control group. The overall MD of the VAS score showed that there was no significant difference between the hyoscine or placebo group [MD: -0.28 (-1.08, 0.52), (p=0.49)]. Pooled analysis was homogeneous (p=0.24); I^2=29%, as shown in Figure 3.

2. Need for postoperative analgesia

The need for postoperative analgesia was reported by all studies. The overall MD favored neither the hyoscine nor the placebo [MD: 0.43 (0.16, 1.14), (p=0.09)]. Pooled analysis was

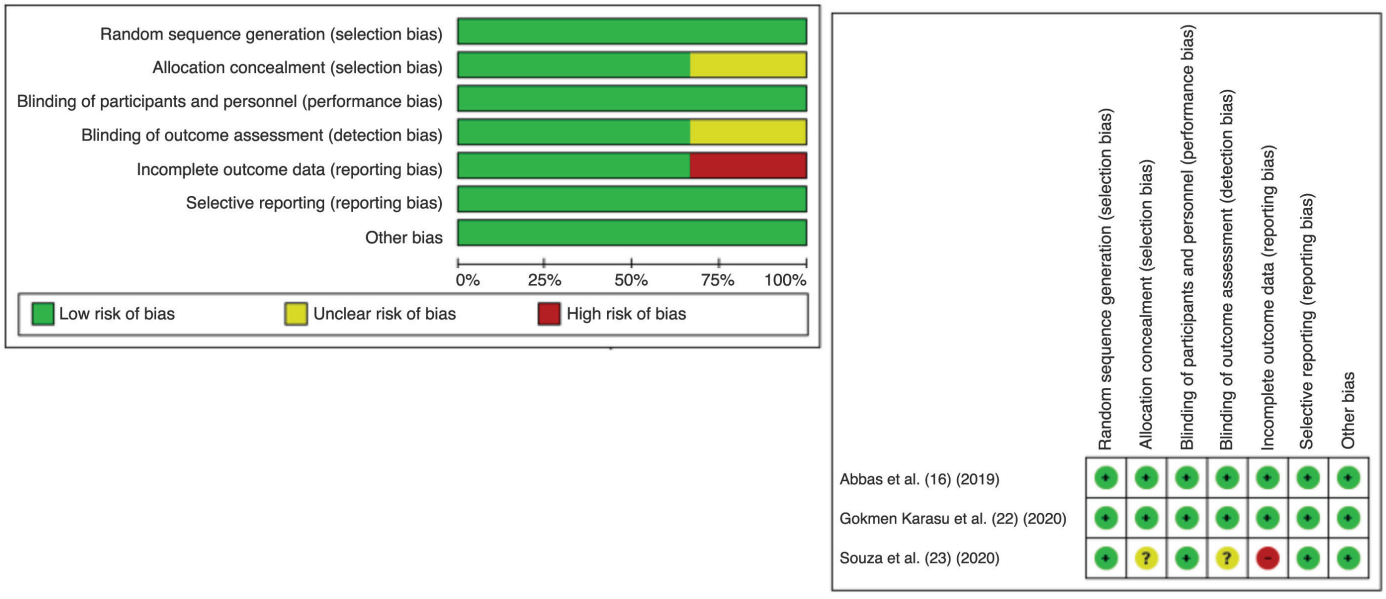

Figure 2. Summary and graph of risk of bias of the included studies

Table 1. Demographic and clinical characteristics of study participants in the groups receiving hyoscine and those receiving placebo

Study ID	Age, years (mean ± SD)		BMI kg/m² (mean ± SD)		C-section, n (%)/(mean ± SD)		Chronic pelvic pain, n (%)		Weight kg, (mean ± SD)		Height cm, (mean ± SD)	
	HBB	PL	HBB	PL	HBB	PL	HBB	PL	HBB	PL	HBB	PL
Abbas et al. (16)	29.81±6.41	30.65±6.91	24.68±2.12	23.95±2.41	9 (20.9)	10 (23.3)	6 (14)	5 (11.6)	NR	NR	NR	NR
Gokmen Karasu et al. (22)	36.2±7.1	37.1±6.3	26.1±5.7	25.9±5.7	5 (16.5)	5 (16.50)	NR	NR	69.3±13	66.1±14.1	163.4± 6.7	159.7±4.9
Souza et al. (23)	48.4±12.6	50.3±10.4	30.1±10.4	31.2±6.9	0.6±0.9	0.6±0.8	15 (6.90)	14 (6.4)	75.6±16.6	79.3±17.9	159±6	160±8

Data are reported as mean ± SD or n (%).
NR: Not reported, HBB: Hyoscine-N-butyl bromide, PL: Placebo, BMI: Body-mass index, SD: Standard deviation

Table 2. Indications of office hysteroscopy for patients in each of the three included studies, stratified by those receiving hyoscine or those receiving placebo

Study ID	Abnormal uterine bleeding		Recurrent miscarriage		Infertility	
	HBB	PL	HBB	PL	HBB	PL
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
Abbas et al. (16)	6 (14)	9 (20.9)	4 (9.3)	6 (14)	33 (76.7)	28 (65.1)
Gokmen Karasu et al. (22)	NR	NR	NR	NR	NR	NR
Souza et al. (23)	50 (23)	52 (24)	2 (0.9)	2 (0.9)	10 (4.6)	5 (2.4)

Data are reported as frequency (%).
NR: Not reported, HBB: Hyoscine-N-butyl bromide, PL: Placebo

heterogeneous (p=0.01; I^2=76%) as shown in Figure 4A. We solved the heterogeneity by the exclusion of Souza et al. (23) (p=0.69; I^2=0%). The pooled analysis after exclusion of Souza et al. (23) significantly favored the hyoscine group [MD: 0.26 (0.16, 0.43) (p<0.01)]. Figure 4B shows the recalculated results of the analysis after one study was excluded (23).

3. Procedure time

Two studies (16,22) reported the procedure time. The combined effect estimate did not show any statistically significant difference between hyoscine and placebo [MD: -0.66 (-2.77, 1.44) (p=0.54)]. Pooled analysis was heterogeneous (p=0.01; I^2=83%) as shown in Figure 5. Heterogeneity could not be solved by the exclusion of one study.

Discussion

Previously published clinical trials reported contradictory results, Abbas et al. (16) and Gokmen Karasu et al. (22) showed that hyoscine significantly reduced postoperative

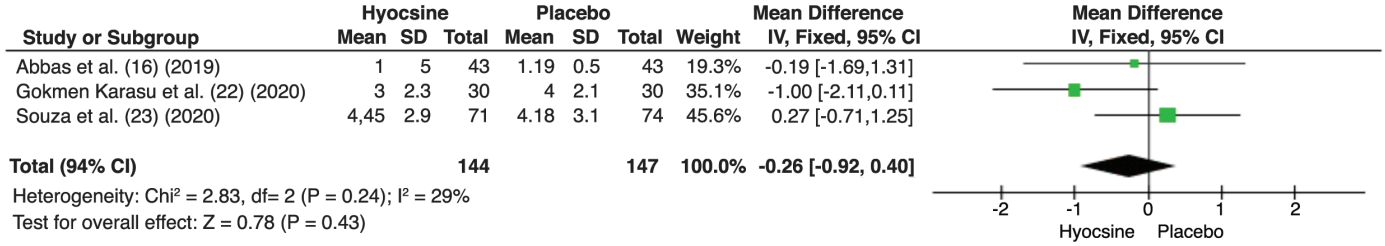

Figure 3. Forest plot for the analysis of VAS score for pain
SD: Standard deviation, CI: Confidence interval, VAS: Visual analogue scale

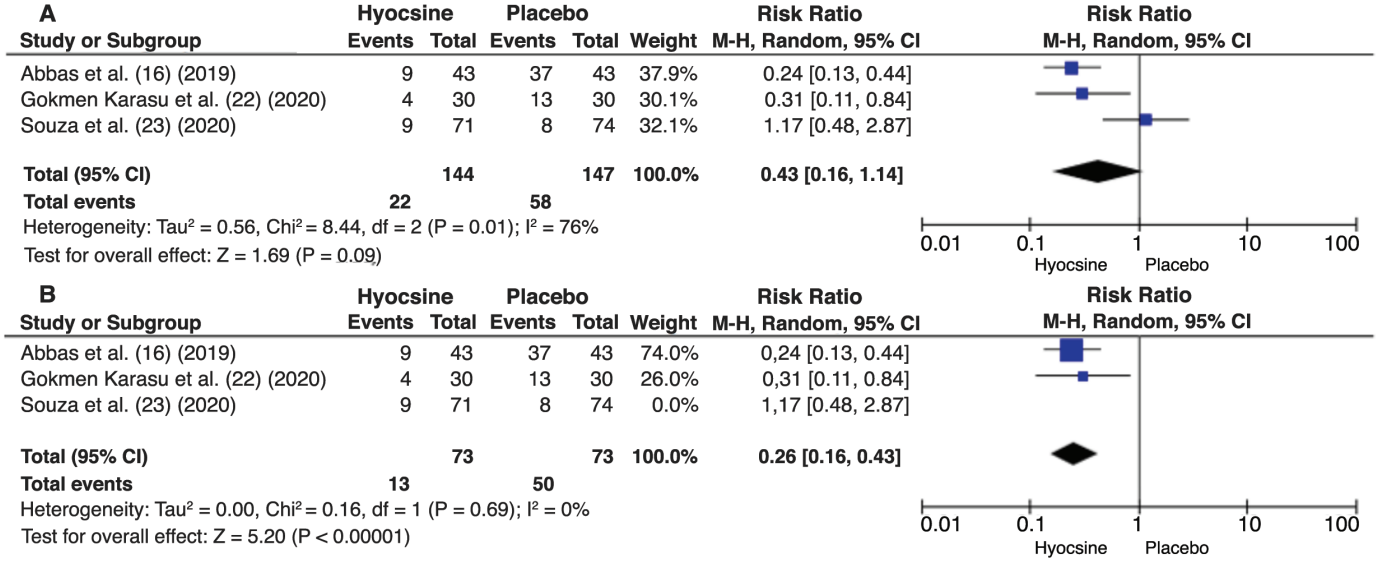

Figure 4. (a) Forest plot for the analysis of the need for postoperative analgesia, and (b) forest plot after removing Souza et al. (23) to solve for heterogeneity
CI: Confidence interval

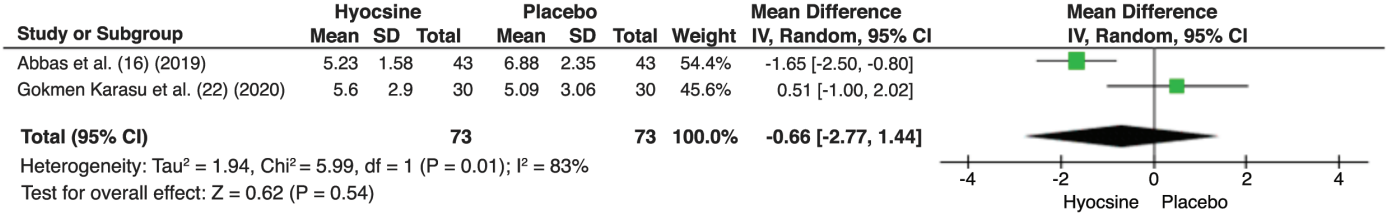

Figure 5. Forest plot for the analysis of procedure time
SD: Standard deviation, CI: Confidence interval

analgesia in patients undergoing hysteroscopy, while Souza et al. (23) reported no significant difference. This could be because Souza et al. (23) used half the dose (10 mg) compared with the studies of Abbas et al. (16) and Gokmen Karasu et al. (22), which both used 20 mg in terms of procedure time, Abbas et al. (16) found that hyoscine reduced the procedure time by 1.65 minutes while Gokmen Karasu et al. (22) showed that the procedure time was similar in both arms. As for the pain score reported during OH, these clinical trials reported no significant efficacy of hyoscine in reducing pain (16,22,23). Our meta-analysis failed to find any significant difference between hyoscine and placebo as far as procedure time, VAS pain score, and the need for postoperative analgesia, when all three studies were included.

As a common procedure carried out in many outpatient clinics, OH has a major role in diagnosing many gynecological abnormalities such as abnormal uterine bleeding, congenital anomalies of the uterus, removal of intrauterine devices and endometrial polyps, and visualization of intrauterine adhesions (1,25,26). The procedure is safe, quick, cheap, and does not usually require general or regional anesthesia (27,28). OH has few side effects reported by patients, of which pain is the most common (29,30). The prevailing explanation as to why pain might arise from the procedure is that cervical dilatation and uterine distension cause more pain to the patient than normal vaginal manipulation (31).

It has been suggested that hyoscine reduces pain by inducing cervical ripening and secreting pro-inflammatory cytokines and prostaglandins (32). It has also been tried as an analgesic for pain management after several gynecological procedures, with varying results. Jareethum et al. (11) investigated the efficacy of hyoscine in women undergoing saline infusion sonography and found no significant effect of the drug on pain reduction. Moro et al. (33) administered hyoscine to patients with infertility undergoing hysterosalpingo-contrast sonography and also found no significant effect. Although many pharmacological and non-pharmacological interventions have been used to reduce pain associated with hysteroscopy (34,35), hyoscine is still used uncommonly and with varying efficacy.

Duan et al. (36) showed that carboprost methylate suppository given vaginally before hysteroscopy is an effective method for reducing pain prior to OH. Tagliaferri et al. (37) showed that saline solution as well as carbon dioxide can be used as acceptable media for performing OH, although it was reported that carbon dioxide had more advantages in reduction of pain perception. Compared with oral diclofenac potassium, hyoscine is not as effective and may have more adverse effects. Abbas et al. (16) found that oral diclofenac potassium administration before diagnostic hysteroscopy reduced pain with subsequent easier and shorter procedure duration. A recent meta-analysis revealed that misoprostol may be an effective medication for managing pain associated with the procedure (38).

Major strengths of our analysis include the overall low ROB among the included trials and the homogeneity of data of the outcomes. Only RCTs were included to ensure high-quality evidence according to GRADE. Although all possible RCTs investigating this topic were included, the major limitation of this study was the small sample size and the low number of published clinical trials. Therefore, it is recommended that more trials to combine hyoscine with other medications or at different doses to obtain more robust data should be performed.

Conclusion

In conclusion, based on the limited evidence available from all available RCTs at this point, there is currently no evidence to support the use of hyoscine in OH.

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The role of ultrasound examination in the management of a patient with hemoperitoneum and an ovarian mass: a clinical and diagnostic challenge

To the Editor,

Ovarian metastasis is a rare presentation of endometrial cancer. Moreover, it is a sporadic cause of hemoperitoneum causing severe anaemia (1). Therefore we decided to describe this case of hemoperitoneum resulting in severe anaemia associated with an ovarian mass which was diagnosed as metastasis of endometrial cancer. The aim of this report was to highlight some observations about this case.

We report the case of a 49-year-old, nulliparous woman with abdominal swelling and severe abdominal pain. She presented with fever and tachycardia. Blood tests showed severe anaemia (hemoglobin: 3.8 g/dL). Both transvaginal and transabdominal ultrasound examinations were performed. They showed thickened, vascularized endometrium of 18 mm, irregular myometrial-endometrial junctions, and a large solid tumor in the right side of the pelvis, measuring 100 mm at the largest diameter with a regular external wall. Color Doppler examination indicated that the tumor was richly vascularized. The left ovary appeared normal. Both ascites and free fluid in the pouch of Douglas were also noted, suggesting hemoperitoneum. Immediately after hemodynamic stabilization and blood transfusions the patient underwent surgery. Laparotomy confirmed the presence of hemoperitoneum and of a large right ovarian mass. The right ovarian mass was removed and the frozen section was positive for borderline tumor (Figure 1). Considering the age of the patient and the ultrasound findings, a radical hysterectomy (Morrow & Querleu type A) and bilateral salpingo-oophorectomy were performed. Final histology reported grade 3 endometrioid carcinoma of the endometrium with 88% myometrial invasion; vagina and parametria were infiltrated. The ovarian mass was found to be a metastasis from the endometrial cancer. The left ovary was described as normal to histopathological examination. The patient underwent chemotherapy, radiotherapy and brachytherapy with good clinical response; she was disease free at 28 months follow-up.

This case was notable for the following peculiarities. Hemoperitoneum and severe anaemia are rarely observed in patients with endometrial cancer. Endometrial cancer usually presents with abnormal uterine bleeding, but this was not the manner of presentation in this patient. Ovarian metastasis is also a rare finding in endometrial cancer (2,3) and it is usually bilateral (4), whereas in the present case the metastasis was unilateral. Our results agree with those previously reported by other authors: ovarian metastases from endometrial cancer usually appear as vascularized solid tumor (4).

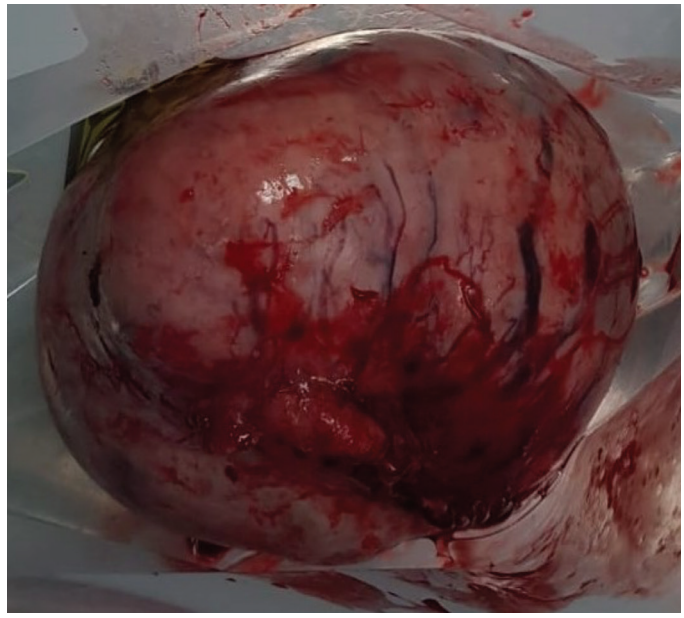

Figure 1. Ovarian mass, send for preliminary histological examination

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This case highlights the difficulties in diagnosing endometrial cancer in the absence of typical symptoms and risk factors. It also emphasizes the key role of ultrasound examination in an emergency setting, in this instance providing guidance to the surgeon to enable planning of the best surgical treatment.

Finally, it should be remembered that only the final histology report will provide the definitive and correct final diagnosis. In our patient, a borderline tumor was suspected at the time of the frozen section, but final histology was positive for an invasive tumor. Borderline tumors require different management from invasive tumors (5). In this case the surgeon performed the correct surgery immediately, because the ultrasound findings suggested a malignant tumor.

We present a patient with endometrial cancer which had metastasized to the ovary and that was discovered because of the unusual presentation of hemoperitoneum and severe anaemia. In addition, the ultrasound examination played an important role in the timely management of this patient.

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Laparoscopic approach for symptomatic pelvic and para-aortic lymphoceles

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Abstract

Description and demonstration of the feasibility of laparoscopic management of symptomatic pelvic lymphocele after surgical staging in gynecological cancer surgery. Step-by-step description of the surgical procedure using pictures and an educational video. Patient gave informed consent for the use of images and the full video article was approved by the Institutional Review Board of the Hospital of Sant Pau. Lymphocele is one of the most common complications of pelvic or lumbo-aortic lymphadenectomy. Although the incidence is variable at 1-58%, around 5-18% of cases are symptomatic. Only symptomatic lymphocele requires treatment, which can be medical or interventional. Drainage is usually performed by guided radiology although a surgical approach has shown a lower rate of recurrence. A 64-years-old woman diagnosed with endometrial carcinosarcoma was staged laparoscopically by pelvic and para-aortic lymphadenectomy. Para-aortic lymphadenectomy was performed using an extraperitoneal approach. Three weeks later she presented with an intense and persistent burning pain, radiating towards the left leg. Computed tomography imaging suggested the presence of a 10x7.6 cm lymphocele adjacent to the left external iliac vessels. Laparoscopy was performed with four-port placement configuration, enabling the identification of a large, bilobed lymphocele, adjacent to the left pelvic wall and left paracolic gutter. Adhesiolysis and identification of main landmarks in the left paracolic gutter and left paravesical fossa was performed as a first step. Peritoneum of each lymphocele was opened in the caudal region and the opening was broadened to facilitate lymph drainage. Owing to the low morbidity and excellent results, we suggest that laparoscopic drainage should be performed as a feasible and useful treatment for pelvic symptomatic lymphoceles.

Keywords: Lymphocele, lymphadenectomy, uterine carcinosarcoma, laparoscopic surgery, oncology

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Introduction

Lymphocele is one of the most common complications of pelvic or para-aortic lymphadenectomy. Although the incidence of subsequent lymphocele varies widely (1-58%), around 4-35% of them are symptomatic (1,2). Lymphocele may cause pain, constipation, urinary frequency or edema of the lower extremities, and can be associated with more severe symptoms, such as infection, hydronephrosis and deep vein thrombosis.

As an interventional approach, percutaneous drainage, which is usually performed by guided radiology, is the preferred method because of its effectiveness, feasibility and low complication rate. However, marsupialization of the cyst is possible when using a surgical approach. Laparoscopic marsupialization has a lower rate of recurrence (3) and has the advantage of minimally invasive approach. Furthermore, there are many factors that may correlate with the presence of lymphocele, such as body mass index, number of obtained lymph nodes and their positivity, degree of lymphadenectomy, the use of postoperative radiation treatment, and the estimated blood loss (>600 mL) (4,5).

We present the case of a 64-year-old woman with a diagnosis of endometrial carcinosarcoma (Video 1). She underwent staging surgery including total hysterectomy along with bilateral adnexectomy and pelvic and lumbo-aortic lymphadenectomy

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by laparoscopy. The number of retrieved nodes were, respectively, 19 and 14 with no evidence of malignant cells. The patient was classified as Stage IB by the International Federation of Gynecology and Obstetrics classification. Para-aortic lymphadenectomy was performed using an extraperitoneal approach, leaving the retroperitoneum open at the end of the procedure to reduce the risk of lymphocele. No tube drainage was inserted after surgery as the evidence suggests that placement of retroperitoneal tube drains has no advantage in preventing lymphocele formation after pelvic lymphadenectomy. To the contrary, a systematic review showed a trend toward an increased risk of symptomatic lymphocele formation in the drained group (5).

Three weeks later the patient presented with intense pain radiating toward the left leg, with a score of 8 out of 10 on the visual analogue scale. The computed tomography (CT) scan suggested the presence of a 10x7.6 cm lymphocele surrounding the left external iliac vessels (Image 1).

The Gynaecology Oncology Committee advised the need for intervention in order to improve her symptoms. Initially, placement of a percutaneous drainage by guided radiology was proposed. However, the patient was very obese and this approach would have been difficult. Thus, surgical treatment was proposed as being more pragmatic.

Laparoscopy was performed with a standard, four-port placement configuration, using a 10 mm optical trocar and three 5 mm accessory trocars placed laterally and suprapubically. As a first step, adhesiolysis and identification of the main landmarks in the left paracolic gutter and left paravesical fossa was performed. The peritoneal surface of each lymphocele was opened in the caudal region (Image 2) and the opening was broadened to facilitate the drainage of the lymph (Image 3).

Total surgical time was fifty minutes and the patient was discharged two days later with improvement of her symptomatology. In the post-operative CT-scan, the cranial lobe of the lymphocele had disappeared, with a residual image of the caudal lobe remaining. However, the patient persisted asymptomatic.

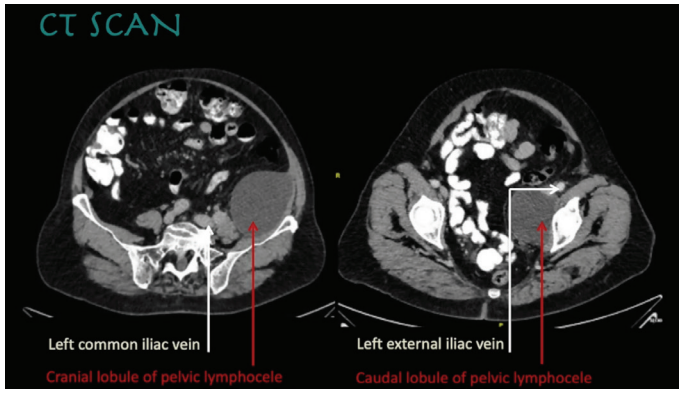

Image 1. Computed tomography scan showing two images suggestive of the presence of pelvic lymphocele

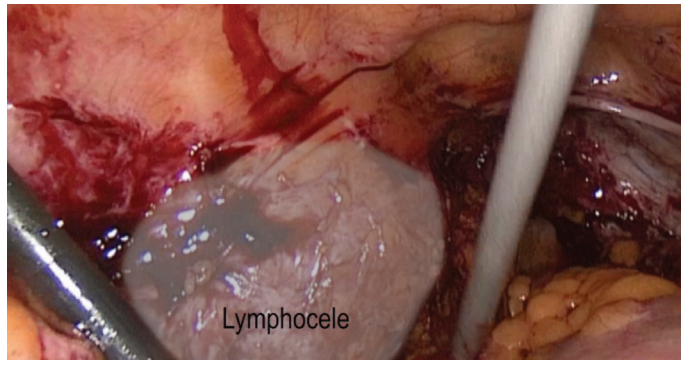

Image 2. Pelvic lymphocele before drainage

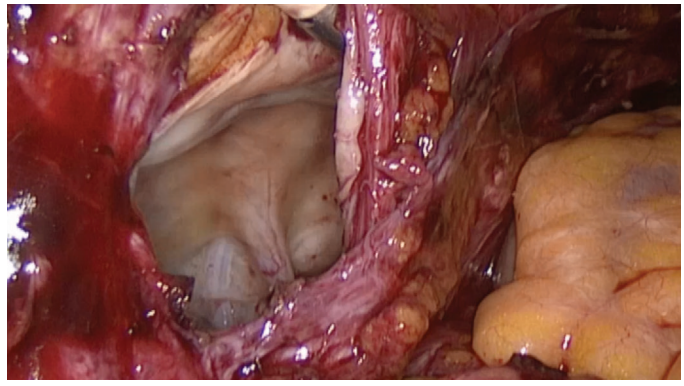

Image 3. Pelvic lymphocele after drainage

Video 1.

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The Blooming phenomenon: a rarity, but a dilemma in hysteroscopic resection of myomas

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Abstract

Modern surgical technologies allow gynecologists to treat most submucosal myomas hysteroscopically by some form of resection. What appears on imaging or direct visualization to be a submucosal myoma can be a single tumor, or may represent multiple smaller myomas appearing as one, compacted together in a typical pseudo capsule. During myoma resection, the effect of the media used to induce distension can vary, depending on the morphology of the myomas. After starting resection, the pressure of the distending media can push truly solitary myomas to somewhat flatten against the uterine wall. However, in the second type of myoma, the fluid can displace the myomas into the uterine cavity, an appearance similar to the blooming of a flower. The tip of the hysteroscope may enter the dissected spaces between the myomas, which impairs the panoramic view. This phenomenon may cause inadequate treatment of the myomas encountered during hysteroscopic myomectomy. In this study, the "Blooming phenomenon" is introduced, and the problems created by this phenomenon and solutions for its management are considered.

Keywords: Leiomyoma, submucosal myoma resection, fibroid, hysteroscopy

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Introduction

Uterine leiomyomas, fibroids or myomas are the most common benign tumors in reproductive-age women in the world (1,2). Submucosal myomas (FIGO type 0, 1, 2) that derive from myometrial cells just below the endometrium (3) are estimated to be the cause of 5-10% of cases of irregular bleeding, pain, subfertility and infertility (4,5). The advancement in endoscopic surgical techniques has resulted in an improved ability to remove submucous uterine fibroids (6,7). At present, the gold standard treatment for submucous myoma is hysteroscopic myomectomy (7). Different techniques and instruments have been introduced to facilitate the removal of submucosal myomas (7,8). Since hysteroscopic morcellator devices, for example the Myosure, and/or other modern interventional and expensive facilities are not widely available, in many hospitals, submucosal myoma removal is still performed using a resectoscope (9). The removal of a submucosal myoma by resectoscope carries a greater risk than other techniques, because of the potential complications related to the procedure, such as cervix laceration, hemorrhage, uterine perforation, or clinical intravasation syndrome (8,10,11). Studies have shown that the outcome of hysteroscopic submucosal myomectomy may be influenced by a number of factors, including the characteristics of the submucous myoma itself (8,12), pseudocapsule fibroid, and by

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the techniques used to remove the myomas (8). The purpose of this article is to address the dilemma that we have termed "the Blooming phenomenon". The Blooming phenomenon may occur during hysteroscopic submucosal myomectomy with loop resection and can be associated with a number of clinical dilemmas and management problems during hysteroscopy that should be discussed.

The Blooming phenomenon

A pelvic sonography can show submucosal myomas in two different ways: a) genuinely solitary (Figure 1A); or b) apparently singular but in fact multiple myomas closely associated and compacted within a typical pseudo capsule (false solitary myoma) (Figure 1B). When submucosal myoma resection is performed for a genuinely solitary myoma, in some cases the pressure of the media used to induce uterine distension can lead to pressing and flattening of the myoma into and against the uterine wall (Figure 2). It may be necessary to reduce the pressure in order to allow the myoma to protrude more into the uterine cavity and become more visible. In the second type of myoma, once the resection begins and the pseudo capsule opens, the pressure of distension fluid entering the spaces between the myomas and the tendency of multiple myomas to disperse outwards when freed from the capsule, can result in the myomas extruding forward and laterally (Figure 3). This is similar to the blooming of a flower when the bud opens and the sepals, the small green leaf-like covering of the buds, are separated and the petals open. Therefore, the pressure of the distending media can produce different effects, depending on the type of submucous myoma (Figure 1).

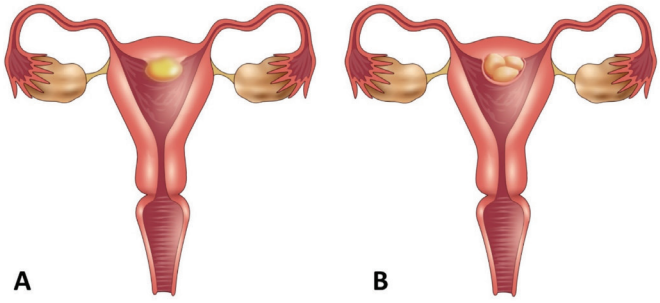

Figure 1. (A) Solitary submucosal myoma; and (B) apparently solitary, but actually multiple submucosal myomas

Another consequence of the Blooming phenomenon is that it reduces the distance between the lens of the hysteroscope and the leading edge of the myomas, which can impair the panoramic view (Figure 4A). Furthermore, the tip of the hysteroscope may enter the dissected spaces between the myomas (Figure 4B). In this latter situation it may be necessary to stop operation before the myomas are completely removed or it may lead to inadvertent resection of the deeper areas of the myometrium and increase the risk of uterine wall perforation.

To manage the Blooming phenomenon, several steps are suggested:

i. The administration of 2-3 months of gonadotropin-releasing hormone agonist pre-operatively, when there is no specific pathology in the endometrium. This will usually decrease the size of myomas, leading to an improved panoramic view. A second consequence of this treatment is endometrial atrophy which can reduce the absorption of fluids during the procedure.

ii. The use of ultrasound and/or magnetic resonance imaging may be helpful in differentiating genuinely solitary myomas from apparently singular myomas that are actually made up of a collection of smaller myomas.

iii. It is best to avoid small vertical or horizontal resections of

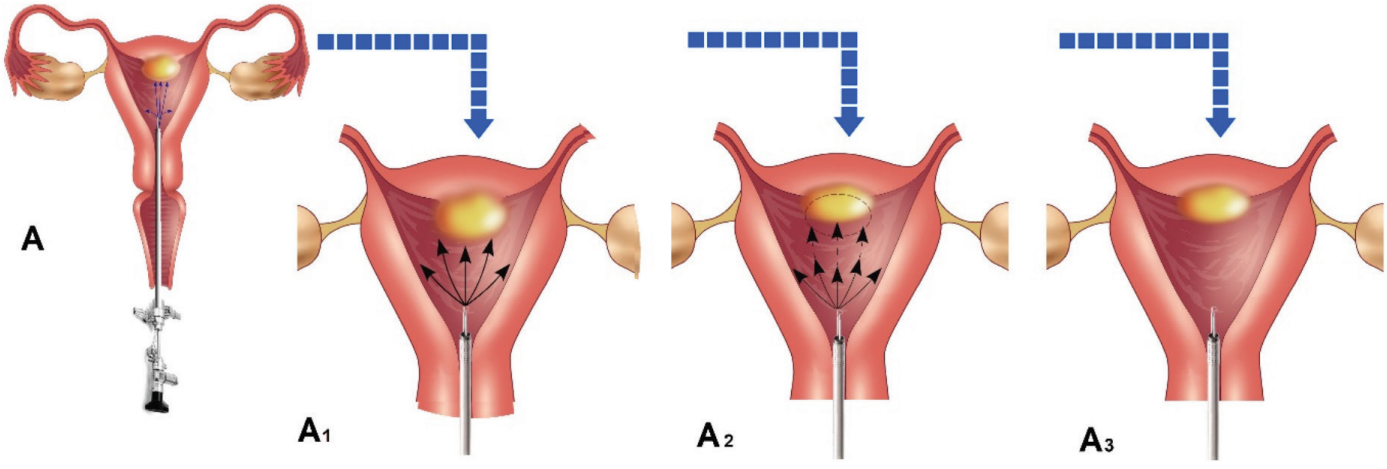

Figure 2. (A) Solitary submucosal myoma; (A_1) arrows show the pressure of the distending media on the myoma; (A_2) pressing and flattening of the true solitary myoma; and (A_3) flattened solitary myoma due to pressure of distending media

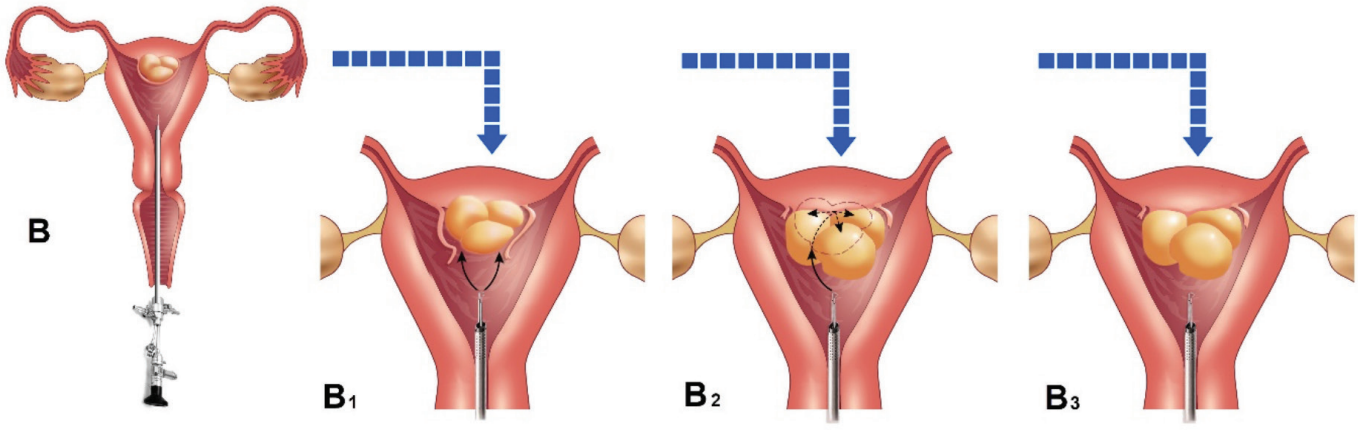

Figure 3. (B) resection of false solitary myoma; (B_1) pseudo capsule opened due to resection; (B_2) fluid entering the spaces between the myomas; and (B_3) release of intracapsular compression leading to extrusion of the multiple small myomas by fluid displacement

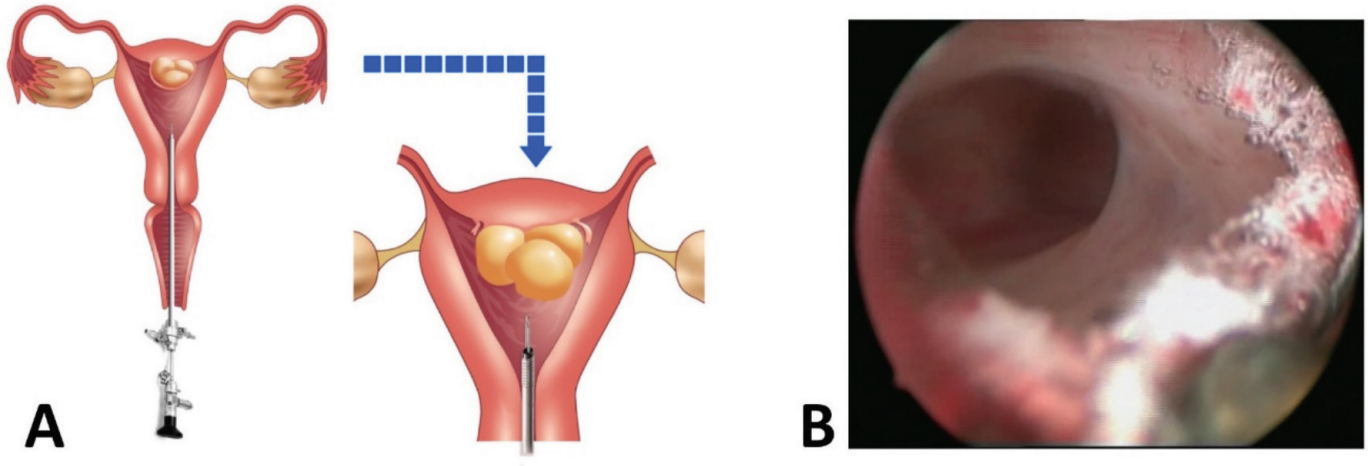

Figure 4. (A) schematic view of dissected spaces between the myomas; and (B) real hysteroscopic view of dissected myoma showing an inadvertent resection of the deeper areas of the myometrium

the myoma (Figure 5A, B, C, D), and it is better to place the tip of the resecting device near to the junction of myoma and the uterine wall and resect obliquely from the base to the tip (Figure 5E). This reduces the possibility that the myomas will protrude into the cavity and limit vision.

In summary, although the Blooming phenomenon is rare, when it does occur it can result in some clinical problems during a hysteroscopic resection. Therefore, surgeons should be aware of the existence of this phenomenon, to prevent potential complications and to know some techniques for the correct management, should they encounter the Blooming phenomenon.

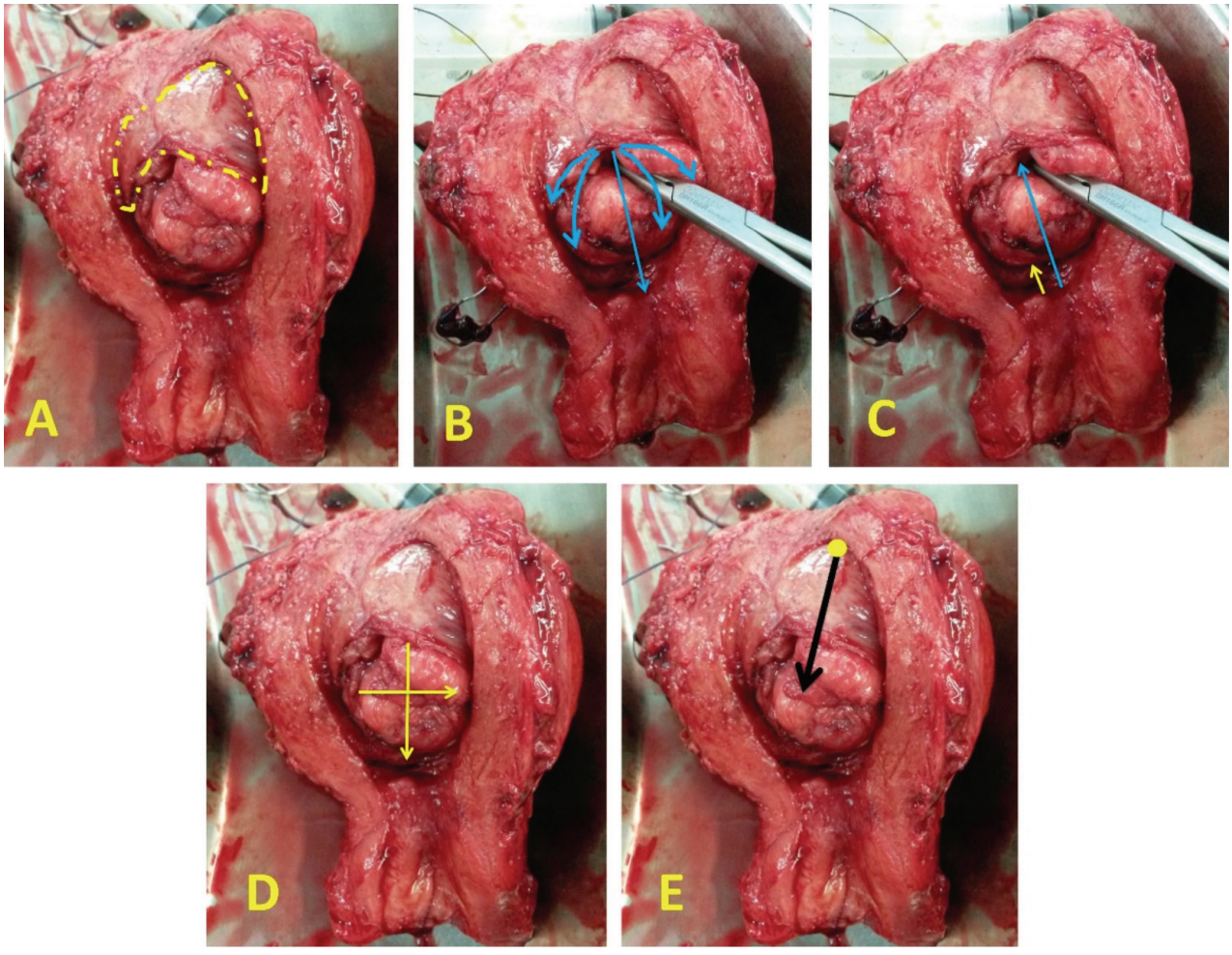

Figure 5. Hysterectomy of the same patient in Figure 4B with failed hysteroscopic myomectomy. (A) the yellow dotted line represents the pseudo capsule of the myoma; (B) blue arrows indicate the directions of protrusions of the myomas after partial resection of pseudo capsule; (C) blue arrow indicates the distance between tip of hysteroscope and myoma before dissection of pseudo capsule, while the yellow arrow demonstrates the reduction of this distance after dissection of pseudo capsule; (D) vertical and transverse resection direction of myoma which should be avoided; and (E) black arrow represents the correct direction of the resection

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Video 1. The Blooming phenomenon during hysteroscopic resection of myoma

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Sclerosing stromal tumor: a rare ovarian neoplasm

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Abstract

Sclerosing stromal tumor (SST) is an extremely rare and distinctive sex cord stromal tumor, which occurs predominantly in the second and third decades of life. SSTs make up 2-6% of ovarian sex-cord stromal tumors. Due to the solid and distinct vascular structure of the tumor, it can be mistaken as a number of malignant ovarian tumors. As this specific neoplasm is very rare, it is not always possible to diagnose the tumor preoperatively with clinical and ultrasonographic findings. Furthermore, histopathological and immunohistochemical analysis does not always confirm the diagnosis. In this case report, clinical findings, histopathological features, and macroscopic appearance during laparoscopy of an SST are presented in a 20-year-old woman with pelvic pain. SST should be considered among the differential diagnosis of women with adnexal masses.

Keywords: Benign ovarian neoplasm, laparoscopy, sclerosing stromal tumor

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Introduction

Sclerosing stromal tumor (SST) is a very rare, benign ovarian tumor, which was first described in 1973 by Chalvardjian and Scully (1). To date, less than 208 cases have been reported worldwide (2). SST is categorized as being one of the sex cord stromal ovarian neoplasms. It can be differentiated from other stromal tumors clinically as well as pathologically (3). Between 2% and 6% of all the sex cord stromal tumors are SST (4). These tumors are commonly seen in patents who are in their second or third decades (3). Pelvic pain, menstrual irregularities and abdominal mass are the most common symptoms and findings. Solid structures in the macroscopic examination of the tumor may be mistaken for malignancy. This may lead to unnecessary radical surgery (5). SSTs are usually unilateral, and well demarcated and recurrences are not reported (3). Histopathologic and immunohistochemical analyses confirm the diagnosis.

The purpose of this case presentation was to show the macroscopic view and the laparoscopic excision of an SST in a 20-year-old woman. To the best of our knowledge, this is the first video article to describe a laparoscopic SST operation.

Presentation of case

A 20-year-old virgin woman attended our outpatient gynecology clinic with the complaint of lower abdominal pain for six months. During the physical examination, an abdominopelvic mass was detected at the right lower abdominal area. A unilateral, heterogenous, cystic mass, originating from the right adnexal area was visualized with ultrasonography. No pathological laboratory findings were reported. All the tumor markers were in the normal ranges. On magnetic resonance imaging a heterogeneous, smooth, contoured mass with fat-intensity areas and solid components was observed in the right adnexal area with measurements of 60x50 mm. Dermoid cyst was considered as a differential diagnosis. The patient was referred to gynecologic oncology. Since malignancy was not primarily considered, laparoscopic cystectomy was planned by the gynecology team. A 10 mm trocar was inserted into the abdominal cavity by direct entry technique from the umbilicus, and a pneumoperitoneum was created. Two

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lateral trocars were placed on bilateral lower quadrants, and one suprapubic trocar was placed in the same plane as one lateral trocar. During the operation, a 60x50 mm sized, multi-lobulated mass with a smooth and intact external surface, apparently originating from the right ovary, was observed. When cut, the internal surface of the mass was grey white to yellowish in color and was solid with a rubbery consistency and contained small cystic spaces. The mass was attached to the ovarian cortex very tightly, and there was a dense blood supply to the mass. It was hard to separate the mass from the ovarian cortex. During the operation, multiple contaminated, whitish viscous tissue pieces, the largest being 4.5x3x2 cm, and the smallest being 1x0.5x0.3 cm were sent for frozen section examination. The result was reported as sex cord stromal tumor (fibroma?), although the definite diagnosis would have to wait for paraffin section examination. The tumor was totally excised and the operation ended. A total operative time of 45 minutes and estimated blood loss of 150 mL were recorded. No intraoperative surgical complications were observed. On postoperative day 1, the patient was discharged from the hospital uneventfully. Histopathologically, the definite result was reported as "SST" (Supplementary Video 1).

The patient was examined one, six and 12 months postoperatively. During the follow-up examinations, the patient reported that her inguinal and abdominal pain had completely resolved. During the follow-up period recurrence was not observed.

Written informed consent was obtained from the patient for publication of this video article and any accompanying images.

Discussion

Approximately 8% of all primary ovarian neoplasms are ovarian sex cord stromal tumors (6). Granulosa cell tumors, fibrotechomas, Sertoli-Leydig cell tumors, steroid cell tumors, and SSTs are categorized as ovarian sex cord stromal tumors (6). Commonly, ovarian sex cord stromal tumors are seen in a single ovary, but rarely they can be detected bilaterally. The youngest patient reported in the literature was 4-years old (7). In our case, the patient was 20 years old and had a unilateral ovarian cyst.

Frequently seen symptoms include menstrual irregularities and pelvic pain (3). There may be masculinization or anovulation due to estrogen and/or androgen secretion (3). In our case there was no clinical virilization and hormone levels and tumor markers were normal.

Macroscopically, SST is a solid, often yellowish mass, varying in size from 3 to 17 cm. SSTs also tend to be well differentiated and usually present with edema and cystic components. The tumor consists of cellular areas with pseudolobular structures surrounded by edematous and collagenous stroma. Hemangiopericytoma-like capillary-rich fields can be detected in these cellular areas (8). Lobule structures consist of two types of cells; spindle-shaped cell secreting collagen and Theca-like cells containing lipids, eosinophilic cytoplasm with vacuoles, and with small dark nucleii with a definite nucleolus (9). It has been reported that inhibin and calretinin are important immunohistochemical markers that help in the diagnosis of ovarian sex cord stromal tumors (5). In our specimen, positive immunohistochemical staining for inhibin and calretinin led us to believe that the tumor originated from stroma.

Differential diagnosis of the SST is essential. Frozen section is crucial for making a distinction between SST and malignant ovarian tumors, because of the similarity in their macroscopic appearance (9). An SST may easily be mistaken for a fibroma or thecoma, both clinically and histopathologically (9). The pattern of the tumor and the patient age will help to differentiate SST from other tumors. Massive ovarian edema may be present with SSTs. In order to eliminate this confusion, compressed ovarian tissue can be identified by palpation of the stroma in the massive ovarian edema (10). SSTs can be treated successfully with unilateral salpingo-oophorectomy or enucleation. There is no local or distant metastasis reported in the literature (10).

Conclusion

As SSTs are rarely encountered, a preoperative clinical and ultrasonographic diagnosis can be challenging. SST should be considered in the differential diagnosis of patients presenting with unilateral, solid cystic, and complex ovarian masses. This tumor has a benign course and good prognosis with conservative surgery.

Supplementary Video 1. Laparoscopic excision of "sclerosing stromal tumor: a rare ovarian neoplasm"

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

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