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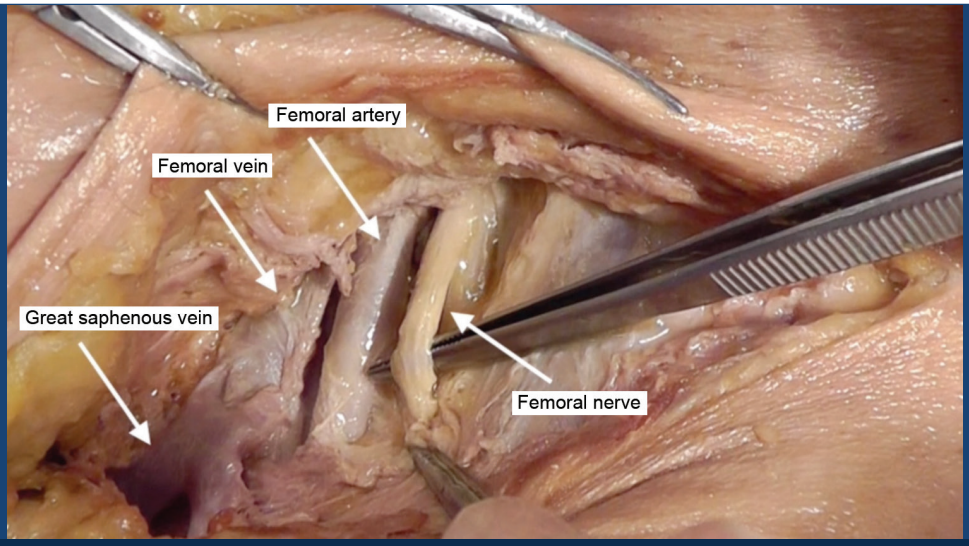

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Aims and Scope

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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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Describe the plan, the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed. In addition to the normal peer review procedure, all randomized controlled trials (RCTs) submitted to the journal are sent to members of a team of professional medical statisticians for reviewing.

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Editorial

Dear Colleagues,

I am delighted to introduce the second issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2021. This issue is consisted of eight articles and one review that we hope you will read with interest.

Magnetic resonance spectroscopy (MRS) can analyze metabolite content in biological fluids with great accuracy using conventional MR imaging. You will read an intersting study determining and comparing metabolite content of growing follicles in patients with polycystic ovary syndrome (PCOS) using MRS.

Premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) are characterized by somatic and psychologic symptoms that arise at the luteal phase of the menstrual cycle and subside with menstruation. You will read a comprehensive review on the subject.

Also you will get the occasion to read a meta-analysis aiming to compare the effect of laparoscopic supracervical hysterectomy (LSH) with endometrial ablation (EA) in terms of general and menstrual-related quality of life in women opting for surgical treatment for abnormal uterine bleeding.

Dear Esteemed Readers,

During the years, the number of submitted papers increased remarkably from all over the world. In this issue, we have made a plan to give most of our space to the publication of Original Articles. This was to publish accepted material without further delay. One of our missions is education and I assure you that we will continue to include education articles, videos and quizes.

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Sincerely,

Prof. Cihat Ünlü, M.D.
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President of TGGF

Anti-phospholipid antibodies in women presenting with preterm delivery because of preeclampsia or placental insufficiency

Rubina Izhar[1], Syed Hasan Ala[2], Samia Husain[1], Sonia Husain[1]

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Abstract

Objective: To assess the proportion of women presenting with preterm delivery because of preeclampsia or placental insufficiency (PREPI) with anti-phospholipid antibodies (APLA).

Material and Methods: This was a prospective cohort study conducted at an obstetrics and gynecology department. Women, aged 20-40 years, with preeclampsia who delivered before 34 weeks were cases while those who delivered before 34 weeks but did not have preeclampsia acted as controls. Both groups had APLA measured at diagnosis and 12-weeks postnatally. Anti-phospholipid antibody syndrome (APS) was diagnosed according to Sapporo's criteria.

Results: The study included 98 cases and 106 controls. Both cases and controls were similar in terms of age, gestational age and parity. The frequency of APS positivity was 17.3% in cases but only 3.8% in controls ($p=0.001$). Cases were more likely to be of Baloch ethnicity (34.7% vs. 11.3%, $p=0.001$), have a history of miscarriage (25.5% vs. 13.2%, $p=0.026$), use aspirin ($p<0.001$) or low molecular weight heparin ($p<0.001$), and be obese ($p<0.001$) than controls. Cases were more likely to have lupus anticoagulant antibodies (82.4% vs. 75%).

Conclusion: Our study confirms a high prevalence of APLA in women who have preterm delivery due to PREPI. An opportunity to screen these women should be made, so that proper counselling can be given and future pregnancies can be managed in an appropriate and timely manner. (J Turk Ger Gynecol Assoc 2021; 22: 85-90)

Keywords: Anti-phospholipid antibody syndrome, preterm delivery, preeclampsia, Sapporo's criteria, screening

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Introduction

Anti-phospholipid antibody syndrome (APS) is an autoimmune disorder associated with the presence of autoantibodies specific for a number of different phospholipid molecules including lupus anticoagulant (LAC), anti-cardiolipin antibodies (ACA) and anti-β2-glycoprotein I (aβ2GPI) antibodies. APS presents as a spectrum of clinical phenotypes, such as thrombosis in the veins, arteries and microvasculature or the sequelae of these thromboses, as well as obstetrical complications (1).

Thrombosis is the hallmark of APS and leads to complications such as recurrent miscarriage and fetal demise in early pregnancy. Late complications comprise pre-eclampsia, preterm delivery before 34 weeks and intrauterine growth restriction due to placental insufficiency.

Preterm delivery for preeclampsia or placental insufficiency (PREPI) is a clinical criterion for APS (2). Preeclampsia leads to high maternal mortality and morbidity and is also implicated in fetal and neonatal morbidity. Worldwide estimates range from 5-10 percent (3). Nulliparity, a family or personal history of preeclampsia, raised body mass index (BMI), advanced maternal age, chronic kidney disease, chronic hypertension and autoimmune disease are known risk factors. However, many risk factors are still under-researched (4). An association between anti-phospholipid antibodies (APLA) and preterm delivery due to placental disease has been proven by several

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case control and cohort studies. The majority of earlier studies have had small sample sizes, used variable definitions of preeclampsia and have frequently included women who develop preeclampsia at term (5-8). Some studies have included low levels of APLA titers, which are not in line with Sapporo's criteria. Therefore the evidence base for this association is not robust and there is a lack of quality studies that prove an association of APLA with pregnancy morbidity (9).

Only one prospective study has addressed this issue to date. However the authors mentioned that the study had a few limitations including that repeat testing was not performed in around half of the women due to a high drop out rate and thus the true proportion with APLA may be higher than that reported. Moreover, the study included women who had delivered before 36 weeks which is outside the specified limit employed by Sapporo's criteria, which is 34 weeks. This would have led to underestimation of the problem by including all women delivering up to 36 weeks as the denominator (10).

Screening for APLA is not widely employed due to the lack of robust evidence, clear consensus and cost constraints (11). Reliable data on the prevalence of APLA in women with PREPI would provide a rationale for establishing a screening policy for these antibodies in such women.

The aim of this study was to prospectively determine the proportion of women with preterm delivery before 34 weeks due to PREPI who also test positive for APLA.

Material and Methods

This was a prospective cohort study, conducted at a department of obstetrics and gynecology, from January 2019 to December 2019. Women aged 20-40 years with preeclampsia who delivered before 34 completed weeks were included as cases. For the study period, both preeclampsia and preeclampsia with severe features was defined as per the American College of Obstetricians and Gynecologists (ACOG) criteria (12). Women who delivered before 34 completed weeks but did not have preeclampsia were included as controls. Women who did not consent, had cervical incompetence, had premature prelabor rupture of membranes, had previous history of cervical trauma, cone biopsy, cervical surgery and women who had known connective tissue disorder were excluded. Patients with a history of chronic hypertension, a history of renal disease or with a history of diabetes mellitus affecting kidneys were also excluded.

After obtaining informed and written consent those with preeclampsia and the controls had APLA measured after delivery. Women were diagnosed with preeclampsia if they had systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure exceeded 90 mmHg. Furthermore, women with acute onset of high blood pressure, with or without proteinuria, along with one or more severe features were included.

For the purpose of the study, placental insufficiency was defined according to the international consensus for the diagnosis of APS (13). Placental insufficiency was defined as delivery due to intrauterine growth restriction, where the estimated weight of fetus was $<10^{th}$ percentile or abdominal circumference was found to be $<5^{th}$ percentile for gestational age. The fetus should have decreased liquor, defined as amniotic fluid index <5 cm or deepest vertical pocket <2 cm, abnormal Doppler ultrasound suggestive of fetal hypoxemia (absent or reversed end diastolic flow), abnormal cardiotocography, or biophysical profile score <6.

For estimation of serum APLA levels, all blood samples were drawn by venipuncture in serum separator tubes. Blood samples were taken for APLA postnatally, and if the first test was positive, repeated 12 weeks later. Serum antibody levels were measured at a reference lab.

Women were diagnosed with APS according to Sapporo's criteria. Sapporo's criteria (13), classify a patient as true APS when at least one of the two clinical criteria are present and at least one of the two laboratory criteria are positive. Clinical criteria include the presence of vascular thrombosis or pregnancy associated morbidity, and laboratory criteria include the presence of medium or high titers of immunoglobulin G (IgG) or IgM ACA or aβ2GPI antibodies by enzyme-linked immunosorbent assay methods or the presence of LAC, based on International Society on Thrombosis and Hemostasis criteria, measured on two or more occasions at least 12 weeks apart (14).

The primary outcome measure was presence or absence of APLA which was defined as any single positive antiphospholipid test, including LAC, ACA IgG (GPL) or IgM (MPL) >40, and aβ2GPI IgG (SGU) or IgM (SMU) >40 (14). As an individual may test positive for more than one antibody at the same time, each antibody was tested separately in all participants.

Statistical analysis

It is reported that women with PREPI have increased odds of having APLA compared to controls (11.5% vs. 1.4%, respectively) (10) With a power of 80% (1-β) and a one-sided 0.05 risk of type 1 error (α), the sample size would need to be 93 patients with preterm delivery in each group to demonstrate a greater proportion of women with PREPI would have APLA. In order to compensate for protocol deviation the sample size was inflated 5% and a minimum of 100 women were to be included in each arm.

Data was analyzed using SPSS, version 16 (IBM Inc., Armonk, NY, USA). Shapiro-Wilk's test was used to assess normality of data distribution. Mean and standard deviations were calculated

for the quantitative variables, such as maternal age, BMI, and parity. Frequencies and percentages were calculated for the qualitative variables, including ethnicity, history of miscarriage, history of aspirin use, low molecular weight heparin use, obesity (BMI >30 kg/m^2), mode of delivery, presence of APS, and LAC and ACA, aβ2GPI antibodies. Effect modifiers were controlled through stratification of maternal age, BMI, parity, ethnicity, history of miscarriage, history of aspirin or low molecular weight heparin use, and obesity, to investigate the effect of these on the outcome variable. Post stratification chi square test was applied taking a p-value of 0.05 as statistically significant.

The study was approved by the Institutional Ethics Committee of Aziz Medical Center (approval number: IEC/AZIZ/334). All participants provided informed consent.

Results

During the study 230 women satisfied the inclusion criteria and were approached for the study. Of these, 12 women refused to participate. Of the remaining 218, 103 (47.25%) women delivered preterm due to PREPI and 115 (52.75%) delivered preterm without preeclampsia. Of those with PREPI, four women were lost to follow up and one refused to undergo the second test for APA and these were excluded. Of the controls, seven women were lost to follow up and two who did not get tested at the reference lab were excluded.

We therefore included 98 cases and 106 controls. The basic characteristics of these women are summarized in Table 1. The mean age of participants was 30.98±3.92 years and had a mean gestational age at delivery of 33.19±1.80 weeks. Most (97, 47.5%) had two children, the largest ethnicity was Sindhi (31.9%) and most delivered vaginally (78.4%). A total of 39 (19.1%) had a history of miscarriage and 41 (20.1%) were obese. There was a history of aspirin use in 49 (24%) women, while 11.7% used low molecular weight heparin. Both cases and controls were similar in terms of age, gestational age in weeks and parity.

Cases were more likely to be Baloch ethnicity (34.7% vs. 11.3%, p=0.001), have a history of miscarriage (25.5% vs. 13.2%, p=0.026) and were using aspirin (p<0.001), low molecular weight heparin (p<0.001), and were more obese (p<0.001) than controls (Table 2). Among the study population, significantly more cases (n=17, 17.3%) had APLA compared with controls (n=4, 3.8%; p=0.001).

ACA were present in 47.1% (8/17) of cases and 50% (2/4) of controls, who tested positive for APA, while LAC was positive in 82.4% (14/17) of cases and 75% (3/4) of controls. Aβ2GPI was positive in 17.6% (3/17) of cases and 25% (1/4) of controls (Table 3).

Discussion

Main findings

The present study assessed the proportion of women with PREPI who had APLA. Our study shows that women with PREPI are more likely to have APLA compared with those who do not have PREPI.

Previous history of miscarriage, aspirin and low molecular weight heparin use and obesity were common in women with PREPI who deliver preterm.

Table 1. Characteristics of study population

Characteristics	Mean ± SD
Age (years)	30.98±3.92
Gestational age in weeks	33.19±1.80
Parity	
1	26 (12.7%)
2	97 (47.5%)
3	55 (27.0%)
4	18 (8.8%)
5	8 (3.9%)
History of miscarriage	
Yes	39 (19.1%)
No	165 (80.9%)
Aspirin use	
Yes	49 (24.0%)
No	155 (76.0%)
Low molecular weight heparin use	
Yes	24 (11.7%)
No	180 (88.3%)
Obese*	
Yes	41 (20.1%)
No	163 (79.9%)
Mode of delivery	
Vaginal delivery	160 (78.4%)
Cesarean section	44 (21.6%)
Ethnicity	
Sindhi	65 (31.9%)
Punjabi	29 (14.2%)
Baloch	46 (22.5%)
Pathani	16 (7.8%)
Urdu-speaking	48 (23.5%)
Group	
PREPI**	98 (48.0%)
Control	106 (52.0%)

*Obesity defined as having a body mass index >30 kg/m^2, **PREPI: Preterm delivery for preeclampsia or placental insufficiency, SD: Standard deviation

LAC was the most common antibody found in women who deliver preterm with PREPI.

Strengths and limitations

The strength of this study is its prospective design and application of Sapporo's criteria of repeat testing and antibody level cut-offs were adhered to, to confirm APS in women with PREPI. International, standard ACOG criteria were used for diagnosing preeclampsia with severe features to guide screening in this group. Our study would aid in strengthening evidence in cases of PREPI for postnatal workup that includes testing for APLA in this high risk group. Follow up of all patients was maintained to ensure the prevalence was not underestimated.

The effect of treatment (aspirin and low molecular weight heparin) was not assessed in these diagnosed cases so it is not possible to comment on the effect of instituting treatment. This could be assessed by future larger trials.

Table 2. Comparison of cases and controls

Characteristics	PREPI**	Controls	p
	Mean ± SD	Mean ± SD	
Age (years)	31.53±3.49	30.46±4.23	0.053
Gestational age in weeks	33.18±1.82	33.20±1.79	0.973
Parity			
1	14 (14.3%)	12 (11.3%)	0.968
2	46 (46.9%)	51 (48.1%)	-
3	25 (25.5%)	30 (28.3%)	-
4	9 (9.2%)	9 (8.5%)	-
5	4 (4.1%)	4 (3.8%)	-
History of miscarriage			
Yes	25 (25.5%)	14 (13.2%)	0.026
No	73 (74.5%)	92 (86.8%)	-
History of aspirin use			
Yes	41 (41.8%)	8 (7.5%)	0.001
No	57 (58.2%)	98 (92.5%)	-
Low molecular weight heparin use			
Yes	21 (21.42%)	3 (2.9%)	0.001
No	77 (78.57%)	103 (97.2%)	-
Obese (BMI >30 kg/m^2)*			
Yes	35 (35.7%)	6 (5.7%)	0.001
No	63 (64.3%)	100 (94.3%)	-
Mode of delivery			
Vaginal delivery	66 (67.3%)	94 (88.7%)	0.001
Cesarean section	32 (32.7%)	12 (11.3%)	-
Ethnicity			
Sindhi	23 (23.5%)	42 (39.6%)	0.01
Punjabi	11 (11.2%)	18 (17.0%)	-
Baloch	34 (34.7%)	12 (11.3%)	-
Pathani	7 (7.1%)	9 (8.5%)	-
Urdu-speaking	23 (23.5%)	25 (23.6%)	-
Antiphospholipid antibodies†			
Yes	17 (17.3%)	4 (3.8%)	0.001
No	81 (82.7%)	102 (96.2%)	-

*BMI body mass index, **PREPI: Preterm delivery for preeclampsia or placental insufficiency, †Antiphospholipid antibodies included lupus anticoagulant (LAC), anticardiolipin antibodies (ACA), and anti-beta-2 glycoprotein I antibodies (aβ2GPI). Presence of antiphospholipid antibodies was defined as any single positive antibody test, including LAC, ACA IgG (GPL) or immunoglobulin (IgM) (MPL) >40, and aβ2GPI IgG (SGU) or IgM (SMU) >40.
SD: Standard deviation

Interpretation

The association of APLA with preeclampsia has been investigated extensively and two meta-analyses have assessed this association. In the first meta-analysis that included 12 studies, criteria for preeclampsia were classified in only six of the included studies (15). Similarly in the second meta-analysis, the association between LAC and preeclampsia was found by five included studies but preterm delivery was not included as an outcome measure in any of the 28 studies that were analysed for the systematic review (16). The only study that investigated preterm delivery suffered due to a lack of follow-up and repeat testing for antibodies, which was denoted as a weakness of the study and potential area for future work (10). Our study rectifies this omission as follow-up was maintained in all cases and repeat testing was ensured.

In a recent study the authors maintained follow up in 52.6% of cases and but noted that the study was limited because of the criteria implemented for preterm delivery and APS (10). The authors included women delivering up to 36 weeks whereas the Saporo criterion gives 34 weeks as the threshold. We included all women who delivered before 34 weeks and therefore ensured that this weakness was also rectified and the evidence base has been strengthened concerning the utility of APLA screening in preeclampsia.

Insufficient evidence in such cases has continued to hinder implementation of testing for APLA in cases of severe preeclampsia (17). The likely benefit of instituting treatment in these cases would more appropriately assess the usefulness of investigation. This is an area for future research. We suggest further studies that look into the role of such intervention in appropriately diagnosed cases.

Table 3. Antibodies in both control and cases

Antibodies*	Group	
	PREPI (n=17)	Control (n=4)
Lupus anticoagulant		
Yes	14 (82.4%)	3 (75.0%)
No	3 (17.6%)	1 (25.0%)
Anticardiolipin antibodies (IgG/IgM)**		
Yes	8 (47.1%)	2 (50.0%)
No	9 (52.9%)	2 (50.0%)
Anti-β2-glycoprotein I antibodies (IgG/IgM)**		
Yes	3 (17.6%)	1 (25.0%)
No	14 (82.4%)	3 (75.0%)

*Any antibody should be demonstrable on at least two occasions separated by 12 weeks, **Both immunoglobulin G (IgG) and IgM were done, due to the small number in control group, the results are not shown individually, PREPI: Preterm delivery for preeclampsia or placental insufficiency

The proportion of women in our population that tested positive for APLA was higher than anticipated. In a study from Utah, 1 in every 9 women, just over 11%, who delivered preterm due to a severe form of preeclampsia had APLA, whereas in our study the proportion was much higher 17.3% (approaching 1 in 5). If repeat testing for confirmation of APS had been performed in the Utah study, a similar prevalence of APLA may have been identified (10). However, on the basis of their findings, these authors claimed that 1 in 9 is sufficiently high to propose screening. We would agree with their statement and add that if the true incidence is closer to 1 in 5, all women who deliver before 34 weeks due to PREPI must undergo screening for APLA to confirm APS. Our study findings also suggest that Pakistani-Asians are a high risk group for this screening.

In our study the cases were more likely to have LAC (n=14, 82.4% vs. n=3, 75%) whereas controls were more likely to have ACL (n=2, 50% vs. n=8, 47%). Hence LAC is more specific for PREPI. This finding was similar to other reports (18).

APS diagnosis requires one lab and one clinical criterion to be positive. Although PREPI is a clinical criterion and thus all women who deliver early because of PREPI already meet the clinical criteria, few women who deliver preterm due to PREPI are ever screened for APLA, and thus the presence or absence of the lab criterion is unknown in most cases (11). Women with APS need special management to prevent further adverse pregnancy outcomes, which cannot be instituted if this opportunity is missed. We counseled and screened all women with PREPI. Our study shows a high enough prevalence to warrant screening in this group.

There is no clear consensus on screening APS in the obstetric population. The Royal College of Obstetricians and Gynaecologists (19) and the Society of Obstetricians and Gynaecologists of Canada (20) do not provide any guidance on APS screening for women with early-onset, severe hypertensive disease of pregnancy whereas the Royal Australian and New Zealand College of Obstetricians and Gynecologists recommends APS screening in these cases (21). In contrast, ACOG recommends against routine screening in the population (22).

Another argument against routine screening is due to lack of data that supports improved outcomes in women with APS. However a recent study showed improved outcomes with targeted therapy (23). We will thus continue to miss an excellent opportunity if we do not provide targeted screening. Our study provides evidence that screening in this population can be fruitful and may lead to better outcomes in future pregnancies.

Conclusion

Our study confirms a high prevalence of APLA in women with PREPI. Testing in this high-risk group can lead to better

fetomaternal and neonatal outcomes. An opportunity to screen these women should not be missed, so that proper counselling can be given and future pregnancies can be managed in an appropriate and timely manner. Screening is insufficient in our region and policy should be implemented to at least target women with PREPI. Given the difference in prevalence reported in this and the American study, it is suggested that the prevalence of APLA in women delivering at or before 34 weeks gestational age due to PREPI should be determined in other populations.

Ethics Committee Approval: *The study was approved by the Institutional Ethics Committee of Aziz Medical Center (approval number: IEC/AZIZ/334).*

Informed Consent: *All participants provided informed consent.*

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Abnormal uterine bleeding types according to the PALM-COEIN FIGO classification in a medically underserved American community

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Abstract

Objective: To describe the distribution of abnormal uterine bleeding (AUB) type according to the polyp (AUB-P); adenomyosis (AUB-A); leiomyoma (AUB-L); malignancy and hyperplasia (AUB-M); coagulopathy (AUB-C); ovulatory dysfunction (AUB-O); endometrial (AUB-E); iatrogenic (AUB-I); and not yet classified International Federation of Gynecology and Obstetrics classification system in a medically underserved American inner-city population. Our secondary objective was to find an association between risk factors and type of AUB.

Material and Methods: We conducted a descriptive cross-sectional analysis at our outpatient women's health clinic located in the Bronx, New York City, from November 2016 to December 2019.

Results: Among 390 patients, the most common AUB type was AUB-L (n=185, 47.4%), followed AUB-P (n=100, 25.6%), AUB-A (n=55, 14.1%), AUB-O (n=19, 4.9%), AUB-M (n=15, 3.8%), AUB-E (n=14, 3.6%) and AUB-I (n=2, 0.5%). Race was distributed as follows: Hispanic (68.2%), Black (25.9%), Caucasian (3.3%), and Asian (2.1%). Comorbidities included hypertension (36.4%), diabetes (15.6%), and thyroid disease (6.9%). The median age at diagnosis was significantly higher in AUB-M (59 years old, $p<0.001$), AUB-P (52.5 years old, $p<0.001$), AUB-E (51.5 years old, $p=0.001$) compared to AUB-L (46 years old). The median body mass index (BMI) was significantly higher in AUB-E (34.2 kg/m^2, $p=0.048$) and AUB-O (32.6 kg/m^2, $p=0.038$) compared to AUB-L (30 kg/m^2). Race was equally distributed among the AUB types. AUB-M (66.6%, $p=0.002$), AUB-E (57.1%, $p=0.022$), AUB-P (47%, $p<0.001$), and AUB-A (30.8%, $p<0.001$), had statistically significantly more cases of hypertension compared to AUB-L (28.1%). AUB-P (27%, $p<0.001$), AUB-M (26.6%, $p=0.025$), AUB-E (35.7%, $p=0.001$) and AUB-A (9%, $p<0.001$) had more patients with diabetes mellitus (DM) than AUB-L (3.3%).

Conclusion: In an American population of medically underserved patients, the most common cause of AUB was leiomyoma and the most common race was Hispanic. Women with AUB-L were younger, with lower BMI, and with fewer cases of hypertension and DM when compared to other types of AUB. (J Turk Ger Gynecol Assoc 2021; 22: 91-6)

Keywords: AUB, PALM-COEIN, abnormal uterine bleeding, myoma, polyp

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Introduction

Abnormal uterine bleeding (AUB) is a common medical condition experienced by approximately one-third of women in their lifetime (1,2). AUB is a broad term that describes irregularities in the menstrual cycle involving frequency, duration, and volume of flow outside of pregnancy (3). AUB is diagnosed in both inpatient and outpatient settings, accounting for up to 70% of consultations with gynecologists (4). The International Federation of Gynecology and Obstetrics (FIGO) created a reproducible classification system for AUB in 2011 (5). The nomenclature for this spectrum is commonly known by the acronym polyp; adenomyosis; leiomyoma; malignancy and hyperplasia; coagulopathy; ovulatory dysfunction; endometrial;

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iatrogenic; and not yet classified (PALM-COEIN). The etiologies correlate to structural disorders such as endometrial/uterine polyp (P), adenomyosis (A), leiomyoma (L), malignant lesions of the uterine body (M); and non-structural disorders such as coagulopathies (C), ovulatory dysfunction (O), endometrial dysfunction (E), iatrogenic (I), and not yet classified (N) (5).

AUB can lead to severe anemia and other medical complications and can significantly impact the patients' quality of life. Several medical and surgical therapies are available, and they are personalized depending on the acuity and severity of the AUB. Hormonal medications, endometrial ablation, hysteroscopic surgery, hysterectomy, and uterine artery embolization are some of the most commonly employed therapeutic options. This common disorder has high direct and indirect costs to the economic system. Frick et al. (6) reported financial losses greater than $2,000 per patient annually due to absence from work and home management costs. Due to the excessive burden that this disorder places on both patients and healthcare systems, it is important to completely describe occurrence and risk factors associated with AUB in all populations, including the women most at risk because they live in underserved areas of the nation.

Other studies have demonstrated the disparities of care which occur in ethnic minorities and the access and management given in gynecological pathology in the USA (7-9). Descriptive studies of AUB subtype distribution in a population in the USA have never been performed. Our aim was to characterize the subset of patients who presented with AUB according to the FIGO classification in an underserved American metropolitan center.

Material and Methods

A descriptive cross-sectional analysis was conducted in an outpatient women's health clinic located in a medically underserved area of The Bronx, New York City, from November 2016 to December 2019. Our center is located in a high-volume, hospital-based clinic that serves 31% of the health care visits in the community where there is fewer than one primary care physician for every 4,000 people (10). The location has allowed access to a unique demographic of New York City's population, with some of the city's most ill and impoverished concentrated in its surrounding neighborhoods.

The study was approved by the Ethics Committee New York Health and Hospitals/Lincoln Institutional Review Board (approval number: N° 19-034). Informed consent was not required because of the retrospective nature of the study, the research involved no more than minimal risk to the subjects and the waiver did not adversely affect the rights and welfare of the subjects.

Pregnant patients were excluded. Patients with AUB, defined as any variation from normal bleeding patterns lasting for a period of at least six months, were included. The AUB was further characterized according to the FIGO PALM-COEIN classification. The diagnosis was based on one or more of these investigations: history, physical examination, sonogram, hysteroscopy, dilatation and curettage, endometrial biopsy, and serum analyses. In addition, the following risk factors were recorded: age, body mass index (BMI), race and comorbidities.

Statistical analysis

Statistical analyses were performed using SPSS for Macintosh, version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as median and interquartile range (25^{th}-75^{th} percentile). Categorical variables were expressed with number of cases/totals. The normality of the data was checked with the Kolmogorov-Smirnov normality test. None of the variables were normally distributed. Therefore, continuous variables were tested with the Mann-Whitney test and categorical variables with the chi-square test. A p-value <0.05 was considered statistically significant.

Results

Three hundred and ninety women were included in the study. The demographic characteristics of the study population, according to the FIGO AUB classification, are shown in Table 1. AUB-L was found to be the most common AUB type (n=185, 47.4%), followed by AUB-P (n=100, 25.6%), AUB-A (n=55, 14.1%), AUB-O (n=19, 4.9%), AUB-M (n=15, 3.8%), AUB-E (n=14, 3.6%) and AUB-I (n=2, 0.5%). No cases of AUB-C and AUB-N were identified.

The median age was 47 (42-53) years old. The median BMI was 30.4 (27.6-34.9) kg/m^2.

Race was distributed as follows: Hispanic (n=266, 68.2%), Black (n=101, 25.9%), Caucasian (n=13, 3.3%), and Asian (n=8, 2.1%) (Figure 1). Comorbidities were distributed as follows: hypertension (n=142, 36.4%), diabetes (n=61, 15.6%), and thyroid disease (n=27, 6.9%) (Figure 2).

Table 2 shows the comparison of the minority AUB types to the majority type (AUB-L) in regard to BMI, age, and chronic medical conditions. The median age was significantly higher in AUB-P (52.5 years old, p<0.001), AUB-M (59 years old, p<0.001), AUB-E (51.5 years old, p=0.001) compared to AUB-L (46 years old). The median BMI was significantly higher in AUB-O (32.6 kg/m^2, p=0.038) and AUB-E (34.2 kg/m^2, p=0.048) compared to AUB-L (30 kg/m^2). Race and thyroid disease were equally distributed among the AUB types.

AUB-M (10/15, 66.6%, p=0.002), AUB-E (8/14, 57.1%, p=0.022), AUB-P (47/100, 47%, p<0.001) and AUB-A (17/55, 30.9%, p<0.001) had statistically significantly more cases of hypertension compared to AUB-L (52/185, 28.1%). AUB-E

(5/14, 35.7%, p=0.001), AUB-P (27/100, 27%, p<0.001), AUB-M (4/15, 26.6%, p=0.025) and AUB-A (5/55, 9%, p<0.001) had a statistically higher number of patients with diabetes mellitus (DM) than AUB-L (13/390, 3.3%).

Discussion

In our study population, the most common type of AUB was AUB-L, followed by AUB-P, AUB-O, AUB-A, AUB-E, AUB-M and AUB-I. We did not find any case of AUB-C and AUB-N.

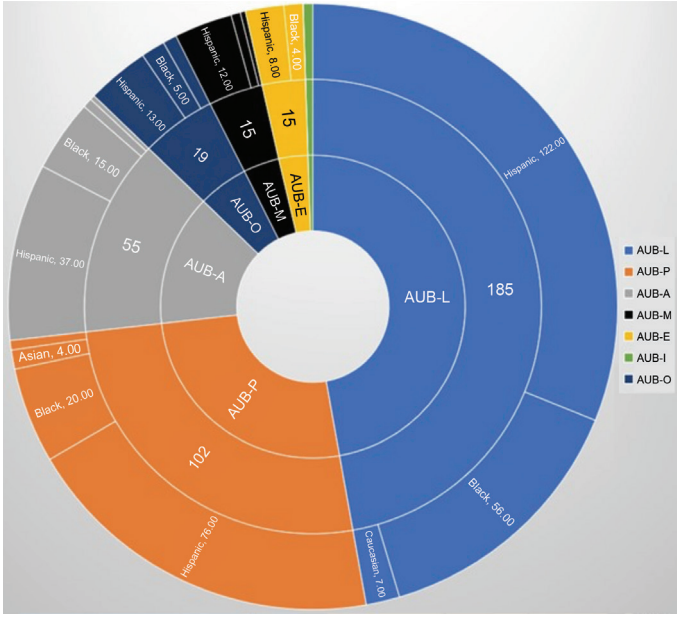

Figure 1. Distribution of races ethnicity (Hispanic, Black, Asian, Caucasian) according to AUB type (AUB-L, M, A, O, P, E, I)

AUB: Abnormal uterine bleeding, P: Polyp, A: Adenomyosis, L: Leiomyoma, M: Malignant lesion, O: Ovulatory dysfunction, E: Endometrial dysfunction, I: Iatrogenic

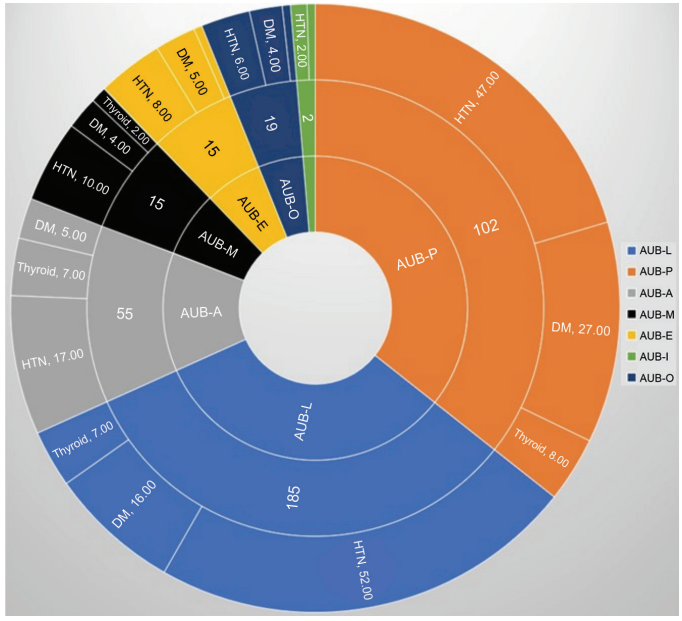

Figure 2. Distribution of comorbidities (HTN, Thyroid Disease, DM) according to AUB type (AUB-L, M, A, O, P, E, I)

HTN: Hypertension, DM: Diabetes mellitus, AUB: Abnormal uterine bleeding, P: Polyp, A: Adenomyosis, L: Leiomyoma, M: Malignant lesion, O: Ovulatory dysfunction, E: Endometrial dysfunction, I: Iatrogenic

Table 1. Main characteristics of study population

Types of AUB	AUB-P (n=100/390, 25.6%)	AUB-A (n=55/390, 14.1%)	AUB-L (n=185/390, 47.4%)	AUB-M (n=15/390, 3.8%)	AUB-O (n=19/390, 4.9%)	AUB-E (n=14/390, 3.6%)	AUB-I (n=2/390, 0.5%)
Age (years)	52.5 (43.5-59.7)	45 (42-49)	46 (42-49)	59 (52-66)	47 (39-50)	51.5 (45.2-63)	52
BMI (kg/m^2)	31.1 (28-35.7)	29.3 (26-34.3)	30.1 (27.4-33.6)	31.2 (27.4-43)	32.6 (29.9-39.6)	34.2 (28.4-41.2)	28.8
Hispanic (n=266/390, 68.2%)	73/266, 27.4%	37/266, 13.9%	121/266, 45.5%	12/266, 4.5%	13/266, 4.9%	8/266, 3.0%	2/266, 0.8%
Black (n=101/390, 25.9%)	20/101, 19.8%	15/101, 14.8%	56/101, 55.4%	1/101, 1.0%	5/101, 5.0%	4/101, 4.0%	0
Asian (n=8/390, 2.1%)	4/8, 50.0%	1/8, 12.5%	0	1/8, 12.5%	0	2/8, 25.0%	0
Caucasian (n=13/390, 3.3%)	3/13, 23.0%	1/13, 7.7%	7/13, 53.8%	1/13, 7.7%	1/13, 7.7%	0	0
Hypertension (n=142/390, 36.4%)	47/142, 33.1%	17/142, 12.0%	52/142, 36.6%	10/142, 7.1%	6/142, 4.2%	8/142, 5.6%	2/142, 1.4%
Diabetes mellitus (n=61/390, 15.6%)	27/61, 44.2%	5/61, 8.2%	16/61, 26.2%	4/61, 6.6%	4/61, 6.6%	5/61, 8.2%	0
Thyroid disease (n=27/390, 6.9%)	8/27, 29.7%	7/27, 25.9%	7/27, 25.9%	2/27, 7.4%	1/27, 3.7%	1/27, 3.7%	1/27, 3.7%

AUB: Abnormal uterine bleeding, P: Polyp, A: Adenomyosis, L: Leiomyoma, M: Malignant lesion, O: Ovulatory dysfunction, E: Endometrial dysfunction, I: Iatrogenic. BMI: Body mass index

Table 2. Comparison of age, BMI, race and comorbidities between patients with AUB-L and the other types of AUB

Types of AUB	AUB-L (185/390, 47.4%)	AUB-P (100/390, 25.6%)	AUB-A (55/390, 14.1%)	AUB-M (15/390, 3.8%)	AUB-O (19/390, 4.9%)	AUB-E (14/390, 3.6%)
Age (years)	45 (38-56)	52.5 (43.5-59.7) p<0.001*	45 (42-49) NS*	59 (52-56) p<0.001*	47 (39-50) NS*	51.5 (45.2-63) p=0.01*
BMI (kg/m^2)	30 (25-39)	31.1 (28-35.7) NS*	29.3 (26-34.3) NS*	32.2 (27.4-43) NS*	32.6 (29.9-39.6) p=0.038*	34.2 (28.4-41.2) p=0.048*
Race (number of Hispanic/Black)	121/367, 32.9% H 56/367, 15.2% B	73/367, 19.8% H 20/367, 5.4% B NS**	37/367, 10.0% H 15/367, 4.0% B NS**	12/367, 3.2% H 1/367, 0.2% B NS**	13/367, 3.5% H 5/367, 1.3% B NS**	8/367, 2.1% H 4/367, 1.0% B NS**
Hypertension (number/total)	53/390, 13.6%	47/100, 47.0% p=0.001**	17/55, 31.0% p<0.001**	10/15, 66.7% p=0.002**	6/19, 31.6% NS**	8/14, 57.1% p=0.022**
Diabetes mellitus (number/total)	13/390, 3.3%	27/100, 27.0% p<0.001**	5/55, 9.0% p<0.001**	4/15, 26.7% p=0.025**	4/19, 21.0% NS**	5/14, 35.7% p=0.001**
Thyroid disease (number/total)	10/390, 2.6%	8/100, 8.0% NS**	7/55, 12.7% NS**	2/15, 13.3% NS**	1/19, 5.3% NS**	1/14, 7.1% NS**

*Mann-Whitney test, **chi-square test. AUB-I was excluded because there were only 2 cases.
BMI: Body mass index, AUB: Abnormal uterine bleeding, P: Polyp, A: Adenomyosis, L: Leiomyoma, M: Malignant lesion, O: Ovulatory dysfunction, E: Endometrial dysfunction, I: Iatrogenic, H: Hispanic, B: Black, NS: Not statistically significant

AUB-L

Leiomyomas are benign monoclonal tumors of uterine smooth muscle tissue. They are the most common pelvic tumor in reproductive age women. The relationship between myomas and AUB is not completely known. Current knowledge suggests that cellular and molecular changes in myomatous tissue may impact on neo-angiogenesis and the release of growth factors (11).

In our study the prevalence of AUB-L was 56.4%.

In 3 previous studies from India (12-14) the prevalence of AUB-L ranged from 24.6% to 30.4%.

We think that the difference in prevalence between our study and the previous studies is related to the differences in demographics of the interested populations.

Indeed, our population was composed mainly of Hispanic and Black women, with a higher prevalence of uterine myomas than the Asian populations described in the previous studies.

AUB-P

Polyps are generated as endometrial outgrowths within the uterine cavity. Most polyps are diagnosed through transvaginal pelvic ultrasound. Histological examination is mandatory in order to exclude any malignancies (15).

In our study, polyp (AUB-P) was responsible for 25.6% of cases (n=100).

Six previous studies from India and 1 from China reported a prevalence of AUB-P ranging from 2% to 16.2% (12-14,16-19).

Our prevalence of AUB-P was higher than the previous studies. The higher prevalence of AUB-P in our population could be caused by a wider use of hysteroscopy in Western countries. Indeed, in our clinic hysteroscopy is readily available and widely used by the providers as one of the first diagnostic modalities of AUB.

AUB-A

Adenomyosis is characterized by the presence of endometrial glands and stroma locally or globally through the uterine musculature, causing hypertrophy of the surrounding myometrium. The pathogenesis of this disorder is not completely known. A diagnostic suspicion for this form of AUB-A can be prompted through a clinical history characterized by dysmenorrhea and diagnostic tools such as ultrasound or magnetic resonance. The definitive diagnosis is however obtained with histological examination after hysterectomy.

Our findings of 14% AUB-A prevalence are in accordance with the previous literature on women from India and China. Indeed, a range of 4.9 to 20% of patients with AUB-A out of a total of patients with AUB is described in the previous literature (12-14,16-18).

AUB-O

We found 19/390 (4.9%) with AUB-O. AUB-O is characterized by a persistent anovulatory state, ultimately leading to irregular endometrial tissue creation, which predisposes to abnormal sloughing of endometrial tissue and bleeding episodes. Ovulation is regulated by circulating levels of estrogen and progesterone, which have been strongly associated with differences in race (20).

Sun et al. (19), in 2018, found that the main cause of AUB in 1053 Chinese women in hospital settings, aged 15 to 55 years, was ovarian dysfunction (n=608, 57.7%). In 2020, Seetha et al. (16) found that ovulation impairment represented the most common cause of AUB, 99 patients (28.2%) after analyzing 350 women of age range 18-45 years old in India. Goel and Rathore (18) in 2016 analyzed 300 patients with no age restriction, from India, with AUB and found AUB-O to be the most prevalent with 85 cases (28.3%). The differences of AUB-O prevalence between our study and the studies by Sun et al. (19), Seetha et al. (16), and Goel and Rathore (18) might be due to differences in population demographics or the settings of the studies with some based in inpatient populations and some in outpatient populations. In fact, only 2.5% of our total study population was of Asian race; the race distribution was not described in Sun et al. (19) and Seetha et al.'s (16), studies but they were both conducted in geographic areas from Asia.

AUB-M

We found 15 women out of 390 (3.8%) affected by AUB-M. AUB-M is mainly caused by cancers of the uterus. Endometrial cancer, the most recurrent cause of AUB-M, represents the fourth most common cancer in the USA after breast, lung and colon cancers (21). Several risk factors have been associated with endometrial cancer, such as race, nulliparity, estrogen therapy, early menarche, delayed menopause and Lynch syndrome. However, obesity, hypertension and diabetes are also implicated as important in the pathogenesis of endometrial cancer. Our results were at the lower end of the range reported in the previous literature where the prevalence of AUB-M ranges from 1.9% to 21.6% (12-14,16-19).

AUB-E

In our study, we found a total of 14/391 (3.6%) with AUB-E.

AUB-E are intrinsic endometrial causes of abnormal bleeding. The diagnosis of AUB-E is reached when other pathologies are excluded either by imaging or histological sampling in the setting of normal ovulatory function.

Gubbala and Mattaparti (17) in 2019 in India studying a population up to 75 years old found that AUB-E was the most common AUB subtype, with 41.9% of the cases. Gubbala and Mattaparti (17) obtained a histopathological sample for each of their patients. On the contrary, in our study the endometrial samples were obtained only at the clinician's discretion and this could be the reason for the different prevalence of AUB-E between our findings and the study of Gubbala and Mattaparti (17).

AUB-I

We found two patients with AUB-I, out of 390 total patients (0.5%). They were both over 50 years old, Hispanic, and both had hypertension. Both of them were on medications to treat breast cancer and the AUB manifested as a side effect of these medications. The AUB-I promptly resolved once the medications were discontinued. Our prevalence result of AUB-I is below the prevalence range found in the literature, ranging from 1% to 11.6% (12-14,16,18,19).

We speculate that the difference between our study and the previous literature may be biased by an under reporting by our providers of AUB-I in our electronic medical records (EMR) system.

Differences of comorbidities among AUB types

In our population, AUB-O and AUB-E had higher BMI than AUB-L. This is likely the results of unopposed estrogen from increased adipose reserves and its effect on both ovulation and the endometrium.

In regard to analysis of age in the population, AUB-P, AUB-M, AUB-E were significantly older than AUB-L. This may be due to the pathogenesis of these specific disorders and the propensity to develop in an older population. The results of our study indicated that hypertension was the most prevalent chronic medical co-morbidity in our patients (n=142, 36.4%), then DM (n=61, 15.6%) and then thyroid disease (n=27, 6.9%) for all the considered groups of AUB type.

AUB-P, AUB-A, AUB-M and AUB-E had statistically significantly more cases of hypertension compared to AUB-L. This might be the effect of hypertension as predominant comorbid condition in advanced ages as these AUB types are seen in that age demographic. The study demonstrated a higher number of AUB-P, AUB-M, AUB-A, and AUB-E patients with DM compared to AUB-L. We speculate that the higher age of AUB-P, AUB-M, AUB-A and AUB-E, compared to AUB-L, likely plays a role in the increased prevalence of DM. Indeed, diabetes and disorders of insulin resistance are chronic medical conditions seen more in geriatric and obese populations. From our analysis, race differences and distribution of thyroid disease were equally distributed among the AUB types.

Study Strengths and Limitations

One of the strengths of our study is that it is the first study to describe the distribution of AUB subtypes according to the FIGO PALM-COEIN classification in an urban underserved American population, to our knowledge. The absence of AUB-C and AUB-N in our population might be biased by limitations of our EMR system. The study was also limited in the range of comorbidities which could be assessed in relation to the AUB types. Due to limitations in how these are able to be analyzed

in the EMR system, only some of the medical comorbidities (hypertension, diabetes and thyroid disease) were collected.

The comparison between this study and other previous studies was difficult in analyzing the distribution of risk factors among AUB types, as they were all heterogeneous regarding age range, time frame, setting, associated factors and diagnostic investigations.

Conclusion

This study serves as a pilot analysis into prevalence of AUB types and chronic medical conditions in minority populations of an inner-city population in the USA. Our finding revealed that leiomyoma represented the most common cause of AUB while the most common race was Hispanic. Moreover, women affected by leiomyoma were younger, with lower BMI and with lower incidence of hypertension and DM when compared to other types of AUB. Future studies are needed to stratify the management options for AUB in these select populations.

Ethics Committee Approval: *The study was approved by the Ethics Committee New York Health and Hospitals/Lincoln Institutional Review Board (approval number: 19-034).*

Informed Consent: *This was not required because of the retrospective nature of the study; the research involves no more than minimal risk to the subjects and the waiver will not adversely affect the rights and welfare of the subjects.*

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

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Systematic review, meta-analysis and statistical analysis of laparoscopic supracervical hysterectomy vs. endometrial ablation

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Abstract

Objective: This meta-analysis aimed to compare the effect of laparoscopic supracervical hysterectomy (LSH) with endometrial ablation (EA) in terms of general and menstrual-related quality of life in women opting for surgical treatment for abnormal uterine bleeding.

Material and Methods: Sources searched included PubMed, Cochrane library, Scopus, and Web of Science for relevant clinical trials. Main outcomes of interest included: quality of life assessed using medical outcomes survey short form-36 (SF-36), (SF-12), operation time, time from operation to discharge, pain, fever, and hemoglobin level. Screening and data extraction were performed independently and the analysis was conducted using Review Manager Software v5.4.1.

Results: Four clinical trials were included. Results of SF-12 score showed that there was no significant difference between the LSH and EA groups for either mental or physical component score overall mean difference (MD): -4.15 (-16.01, 7.71; p=0.49) and MD: 2.67 (-0.37, 5.71; p=0.08), respectively. Subgroup analysis of the SF-36 showed that only two components, general health and social function, were significantly improved in the LSH group (p<0.01) while the other six sub-scores did not differ between groups. The overall MD significantly favored the EA group for: operation time [MD: 72.65 (35.48, 109.82; p=0.0001)], time from operation to discharge [MD: 13.61 (3.21, 24.01; p=0.01)], hemoglobin level outcome [MD: 0.57 (0.40, 0.74); p<0.01], and pain score [standardized MD: 0.46 (0.32, 0.60; p<0.01)].

Conclusion: LSH has better outcomes for quality of life. This includes patient indicated responses to social health, general health, and superior hemoglobin levels at all measured points postoperatively. EA, however, was consistently associated with less operative time, a shorter hospital stay and is also considered by the authors to be a more minimally invasive technique which can also result in satisfying outcomes. (J Turk Ger Gynecol Assoc 2021; 22: 97-106)

Keywords: Supracervical, supracervical hysterectomy, endometrial ablation, abnormal uterine bleeding, systematic review

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This Manuscript has been reviewed by the Institutional IRB Board at Marchand Institute and was found to be exempt from IRB review (September, 2020).

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Introduction

Abnormal uterine bleeding (AUB) is one of the most common gynecological problems that affects a quarter of women in the United States (1). It significantly impairs the quality of life of many women and accounts for a fifth of all hospital gynecology referrals (2). Menorrhagia is subjectively defined by some authors as excessive cyclic menstrual bleeding occurring over several consecutive cycles (3) and is objectively defined as a menstrual blood loss of 80 mL or more per menstrual cycle by other authors (4). Only 34% of females complaining of menorrhagia reported bleeding of more than 80 mL (5). Therefore, there is a discrepancy between women's perception of their menstrual bleeding and objective measures of the amount of blood loss (6). Endometrial polyps, adenomyosis, and fibroid(s) are common structural abnormalities of the uterus, which are associated with menorrhagia (7,8). AUB can also result from an abnormality of the endometrium (hyperplasia or malignancy), a disorder of ovulation, and coagulation defects (9).

Generally, a medical approach is used as the first line for the treatment of AUB, but it has a comparably high failure rate. About 42% of women using an intrauterine hormonal system and 77% of those on oral drugs will need to undergo surgical treatment within five years (10). The two most common surgical approaches for the treatment of AUB are hysterectomy, where the uterus is removed, and endometrial ablation (EA), where the endometrium is thermally destroyed with uterine preservation (11). Abdominal hysterectomy had been the only definitive treatment for AUB, until the development of endoscopy introduced laparoscopic hysterectomy and hysteroscopic EA (12). Most women prefer the laparoscopic approach because it has a less invasive nature and a shorter hospital stay (13). Laparoscopic supracervical hysterectomy (LSH) only removes the uterine body, which is the main source of AUB (14). This is also sometimes referred to as subtotal or partial hysterectomy. In many cases, this procedure offers a less complex surgery that avoids difficult surgical dissection around the cervix and bladder (15). Although controversial, many authors have suggested leaving the cervix and its ligaments can reduce the risk of vaginal vault prolapse, and most authors agree it can reduce the risk of ureteric injury in some cases (16). The availability of newer surgical equipment and the improvements in laparoscopic training have simplified LSH.

EA is an effective and minimally invasive surgical technique that has been developed to remove the entire thickness of the endometrium while sparing the uterine body (17,18). After EA, any residual endometrium is theoretically beneath the created scar, and as a result this prevents further bleeding. Although it improves the general and menstrual-related quality of life, about 20% of women who have this treatment will require a hysterectomy for relief of their symptoms at some point (19). In this meta-analysis, the aim was to compare the effect of LSH with EA in terms of general and menstrual-related quality of life in women opting for surgical treatment for AUB.

Material and Methods

This systematic review and meta-analysis was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (20) and the guidelines reported in the Cochrane Handbook for Systematic Reviews of Interventions (21). Informed consent was not applicable to this study as it is a systematic review performed according to the PRISMA statement protocol.

Search strategy

Online databases which were searched were: Web of Science, Scopus, Cochrane Central, and PubMed, without any restrictions on time or languages. We used the keywords: "ablation", "hysterectomy", "laparoscopy*", and "bleeding" and combined these words by "AND" or "OR" as appropriate for the search.

Eligibility criteria

All studies were included that met the following criteria: 1) patients - women with AUB; 2) intervention - LSH; 3) comparator - EA or resection; 4) outcomes - quality of life assessed using medical outcomes survey short form 36 (SF-36), SF-12, operation time, time from operation to discharge, pain, fever, and hemoglobin level; and 5) study design - only randomized clinical trials (RCTs) were included. Studies were excluded if they contained any of the following criteria: 1) non-RCTs; 2) patients treated with hysterectomy using other techniques; 3) single-armed trials, or with different comparators other than EA or resection; 4) animal trials; and 5) studies that have no available full-text.

Screening of search results

The results of the search were exported into Endnote X8.0.1 (Build 1044), with the removal of duplicates automatically by computer. After that, the studies were screened manually in two steps. Firstly, title and abstract screening. Secondly, full-text screening of the studies which had passed the first step. Articles were included on the basis of the inclusion criteria as defined earlier and studies were removed studies that didn't meet these criteria.

Data extraction and analysis

After the screening step, data were extracted from the selected studies and the outcomes were categorized into two

main groups: 1) baseline and demographic data of patients in each study, including age, body mass index (BMI) (kg/m^2), parity, preoperative hemoglobin level, and incidence of dysmenorrhea; 2) outcomes including quality of life assessed using the medical outcomes SF-36, which consists of eight components (general health, physical function, limitations on life functions (physical), limitations on life functions (emotional), mental health, social function, vitality, and pain), SF-12 which consists of two components [physical component summary (PCS) and mental component summary (MCS)], operation time, time from operation to discharge, pain, fever, and hemoglobin level.

Statistical analysis

We used Review Manager Software (RevMan 5.4.1) to perform our analysis. We expressed dichotomous outcomes using percent and total, while continuous outcomes were expressed using mean difference (MD) and standard deviations, relative to 95% confidence interval. In the case of outcomes reported by different tools or parameters, we used the standardized MD in the analysis. Heterogeneity was assessed using statistical I^2 test and p-value of the chi-square test, where outcomes with I^2 >50%, p<0.1 were considered heterogeneous, while outcomes with I^2<50%, p>0.1 were considered homogeneous. The homogenous data was analyzed using a fixed-effects model, while heterogeneous outcomes were analyzed using the random-effects model.

Quality assessment

The quality of this systematic review and meta-analysis was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines according to the Cochrane risk of bias (ROB) tool for clinical trials (22). The ROB was performed for the included studies. The Cochrane ROB assessment tool includes the following domains: random sequence generation (selection bias), allocation sequence concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other potential sources of bias. The authors' judgment was categorized as "low risk", "high risk", or "unclear risk" of bias.

Results

Summary of included studies

The literature search and references retrieved 127 studies. Twenty studies met the study criteria and were included in full-text screening. Four of these studies met the full eligibility criteria after full-text screening and were included in the meta-analysis. Figure 1 illustrates the PRISMA statement for the literature search. The four acceptable studies included 1018 patients for analysis (23-26), treated by either LSH or EA or resection - grouped as EA hereafter. A total of 517 underwent LSH, while 501 patients underwent EA. The mean age of patients in the LSH group was 47.2+4.77 years, while that of the EA group was 47.55+4.6 years. Table 1 shows a detailed summary of the included participants, their demographic characteristics, BMI, parity, preoperative hemoglobin, and the number of patients who suffered from dysmenorrhea.

Results of risk of bias assessment

The ROB assessment result yielded an overall moderate risk, according to Cochrane's tool (21). Regarding randomization, all studies were at low risk of randomization (23-26). As for allocation concealment, two studies reported insufficient details; therefore, they were categorized as "unclear risk" (25,26). Sesti et al. (24) reported adequate allocation concealment, so it was considered low risk while the study of Cooper et al. (23) showed inadequate concealment of allocation, so it was marked as high risk. Concerning blinding of participants and personnel, all studies did not report sufficient details about blinding of participants and personnel, so they were designated unclear risk, except Sesti et al. (24) that

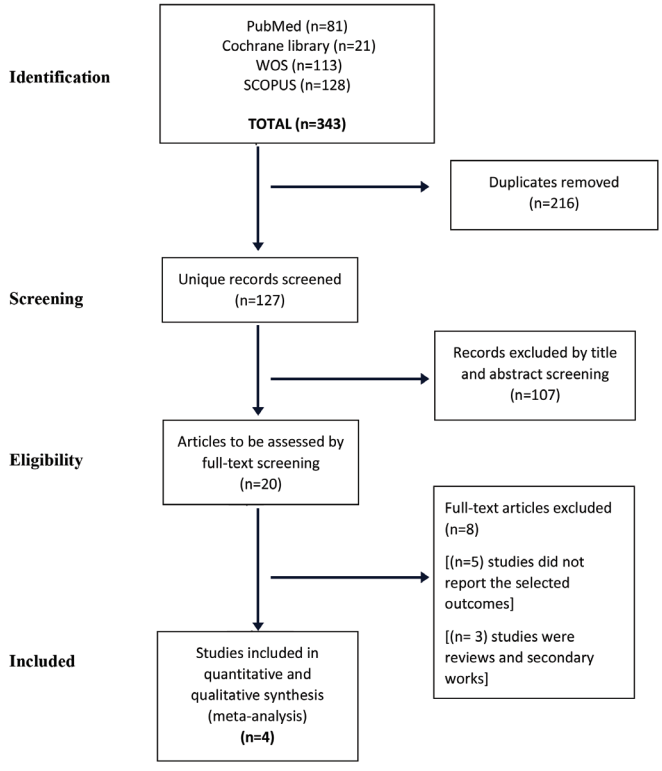

Figure 1. Shows the PRISMA statement of our literature search

PRISMA: Preferred reporting Items for systematic reviews and meta-analyses, WOS: Web of Science

Table 1. A detailed summary of the included participants

Study	Sample size		Age				BMI (kg/m²)				Parity				Pre-operative hemoglobin (g/dL)				Dysmenorrhea			
	LSH	Ablation	LSH		Ablation		LSH		Ablation		LSH		Ablation		LSH		Ablation		LSH		Ablation	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Event	Total	Event	Total
Cooper et al. (23)	309	307	42	5	42	5	29.1	5.6	29	5.3	-	-	-	-	13.1	1.31	13.01	1.26	170	326	160	326
Sesti et al. (24)	34	34	47.5	7.4	47	8.2	24.7	3.3	23.9	2.9	2.2	1.3	2.2	0.8	12.1	1.5	12.1	1.2	-	-	-	-
Zupi et al. (25)	92	89	42.6	4.4	43.2	3.5	34.5	1.9	35.5	1.4	1.9	0.7	1.8	1	9.5	0.9	9.9	0.8	38	92	33	89
Zupi et al. (26)	82	71	57	2.3	58	1.8	26.7	1.1	27.4	2.9	-	-	-	-	-	-	-	-	-	-	-	-

BMI: Body mass index, LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation

reported adequate blinding of participants and personnel, so it was categorized as low risk. As for blinding of outcome assessment, blinding was likely effective in all studies, so they were at low risk (23-26). All studies were at low risk of attrition bias. Moreover, all studies were at low risk of selective reporting. There was no other bias in all studies except for Cooper et al. (23) with unclear risk. A detailed illustration of the ROB of included trials is summarized in Figure 2.

Analysis of outcomes

1. Short form survey-12

Two studies reported the SF-12 outcome (23,26). SF-12 consists of a PCS and a MCS. Regarding MCS, the overall mean difference showed that there was no significant favoring of either group over the other [MD: -4.15 (-16.01, 7.71), (p=0.49)], Pooled data was heterogeneous (p<0.01); I^2: 99%. Regarding PCS, there was no significant difference between both groups [MD: 2.67 (-0.37, 5.71), (p=0.08)]. Pooled analysis was heterogeneous (p=0.001); I^2: 91% as shown in Figure 3. Heterogeneity was not resolvable in either component, either by the leave-one-out method or subgroup analysis because only two studies had reported the outcome.

2. Survey short form-36

SF-36 outcome was reported by two studies (24,25). SF-36 consists of eight components (general health, physical function, limitation on life functions (physical), limitation on life functions (emotional), mental health, social

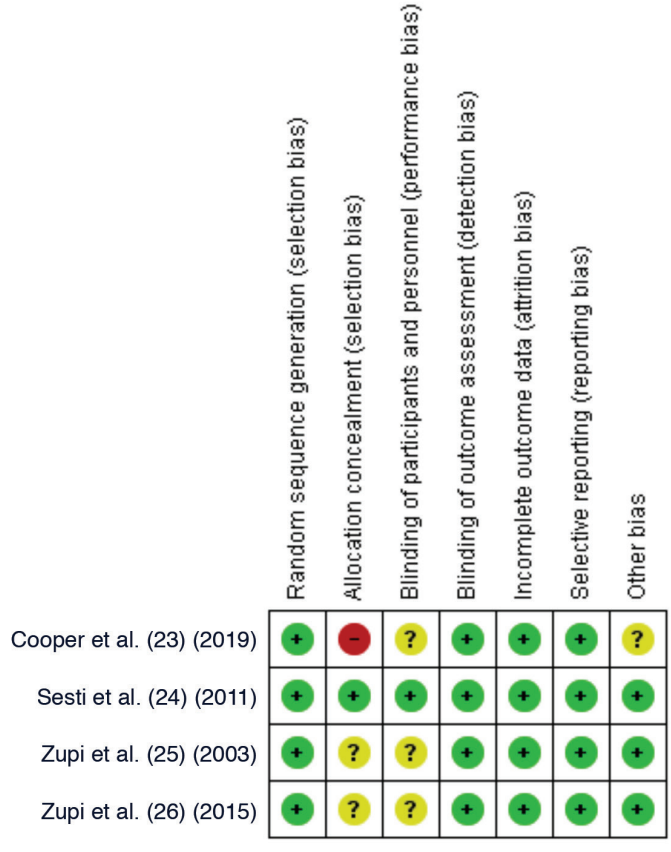

Figure 2. A detailed illustration of the risk of bias of included trials

function, vitality, and pain). Regarding the general health component, the overall mean difference favored the LSH group significantly [MD: 10.25 (7.12, 13.38), (p<0.01)]. Pooled analysis was homogenous (p=0.74); I^2: 0%.

As for physical function component, the total mean difference showed no significant difference between both groups [MD: 10.64 (-8.08, 29.36), (p=0.27)], data was heterogeneous (p<0.01); I^2: 96%. Concerning the limitation on life functions (physical) component, the combined mean difference showed no significant change between both groups [MD: 1.83 (-1.56, 5.22), (p=0.29)]. Pooled studies were homogenous (p=0.41); I^2: 0%.

The analysis of the limitation on life functions (emotional) component yielded no significant variation between both groups [MD: -7.07 (-28.72, 14.59), (p=0.52)]. Data was heterogeneous (p<0.01); I^2: 97%. As for mental health, the comprehensive mean difference showed no significant change between both groups [MD: -5.33 (-21.20, 10.55), (p=0.51)]. The analysis showed heterogeneity (p<0.01); I^2: 96%. With regard to the social function component, the total mean difference favored the LSH group significantly [MD: 16.94 (8.32, 25.56), (p=0.0001)]. Data was heterogeneous (p=0.003); I^2: 88%.

Vitality component analysis indicated no significant change between both groups [MD: 0.22 (-21.73, 22.17), (p=0.98)]. The analysis showed heterogeneity (p<0.01); I^2: 98%. The analysis of the pain component showed that there was no significant favoring of either group over the other [MD: 7.67 (-4.48, 19.83), (p=0.22)]. Pooled studies were heterogeneous (p=0.0002); I^2: 93%. Detailed analysis for each item in SF-36 outcome is shown in Figure 4.

Heterogeneity could not be solved in this outcome either by the leave-one-out method or subgroup analysis because only two studies had reported the outcome.

3. Operation time (in minutes)

Three studies (23-25) reported operation time. The total mean difference favored the EA group significantly [MD: 72.65 (35.48, 109.82), (p=0.0001)]. Pooled studies were heterogeneous (p<0.01); I^2: 98% as shown in Figure 5. Heterogeneity could not be solved either by the leave-one-out method or subgroup analysis.

4. Time from operation to discharge (in Hours)

Cooper et al. (23) and Zupi et al. (25) reported the time from operation to discharge. The analysis favored the EA group over the LSH group significantly [MD: 13.61 (3.21, 24.01), (p=0.01)]. Data was heterogeneous (p=0.02); I^2: 81% as shown in Figure 6. Heterogeneity could not be solved either by the leave-one-out method or by subgroup analysis.

5. Hemoglobin (g/dL)

Sesti et al. (24) and Zupi et al. (25) reported hemoglobin levels postoperatively. Hemoglobin level was significantly lower in the EA group than the LSH group [MD: 0.57 (0.40, 0.74), (p<0.01)]. Pooled analysis was homogenous (p=0.22); I^2: 34% as shown in Figure 7.

6. Pain

Pain outcome was reported by two studies (23,25). The pain score was assessed by two different scales. Therefore, we performed analysis using a standardized MD (SMD). The overall SMD favored the EA group significantly [SMD: 0.46 (0.32, 0.60), (p<0.01)]. The analysis was homogenous (p=0.45); I^2: 0% as shown in Figure 8.

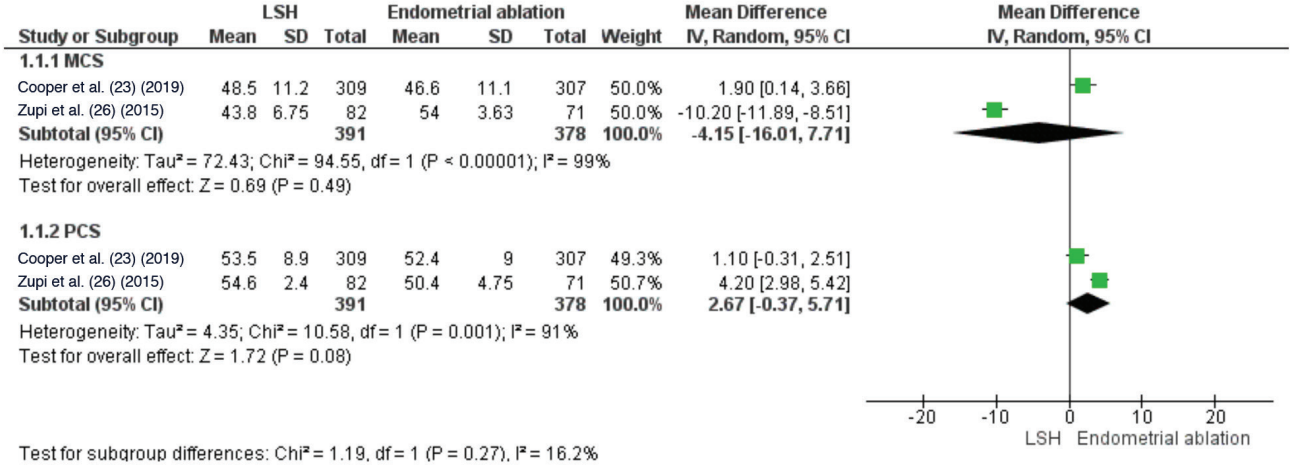

Figure 3. Forest plot for the analysis of SF-12 outcome

LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval, MCS: Mental component summary, PCS: Physical component summary

7. Fever

Two studies (23,25) reported fever outcomes postoperatively. The total mean difference showed no significant variation between both groups [MD: 0.00 (-0.01, 0.02), (p=0.54)]. Data was homogenous (p=0.76); I^2: 0% as shown in Figure 9.

Discussion

Some authors have suggested that when limiting comparison to success rates, that surgery has superior results than different oral medications in the treatment of menstrual bleeding in improving the quality of life of women affected by AUB (27). This is not to say, however, that there is complete agreement across all medical and surgical therapies. For example, Madhu et al. (28), found that there was no significant difference between thermal balloon ablation (TBA) as a conservative surgery and use of a levonorgestrel intrauterine system. While hysterectomy is the only curative treatment that prevents menstrual bleeding 100% in all cases, it also carries a high risk

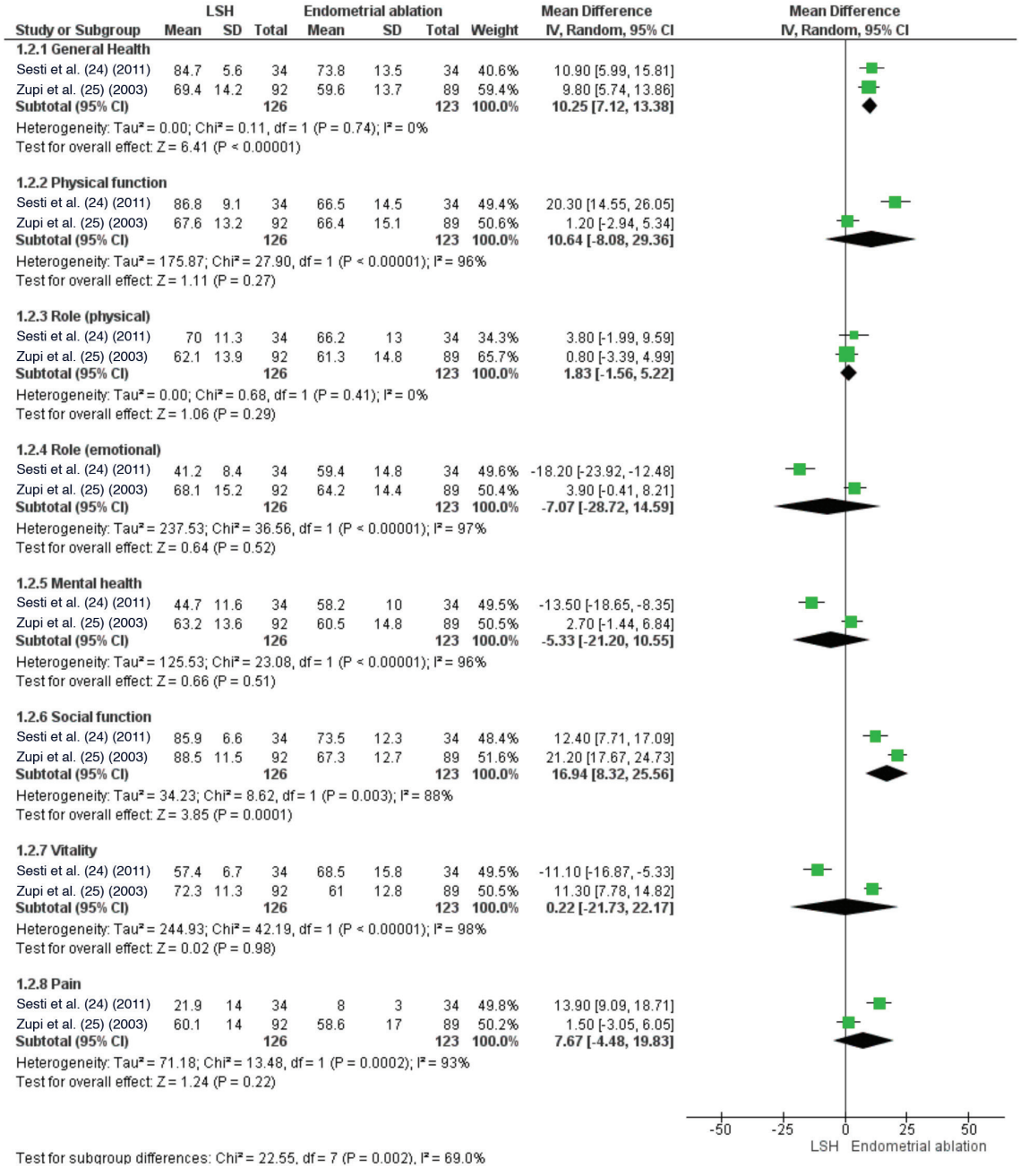

Figure 4. Forest plot for the analysis of SF-36 outcome

LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval

for serious complications. EA has been suggested to be the second line of treatment after failure of medical treatment, rather than a hysterectomy, by several authors (29). The best surgical treatment for AUB, therefore, remains controversial.

In this systematic review and meta-analysis, the aim was to identify the optimal surgical intervention for the treatment of AUB by comparing the outcomes of only LSH and EA or resection. We chose to compare LSH to EA instead of comparing total laparoscopic hysterectomy (TLH) or laparoscopic assisted vaginal hysterectomy (LAVH) because many authors feel LSH is more minimally invasive than TLH and LAVH, because the surgery does not include the creation of a colpotomy or division of the utero-sacral ligaments. Therefore we aimed to compare two very minimally invasive procedures.

In terms of quality of life, it was found that LSH was significantly superior to EA in general health and in social function. However, in other components of the SF-36 there was no significant difference between LSH and EA. Although the analysis didn't

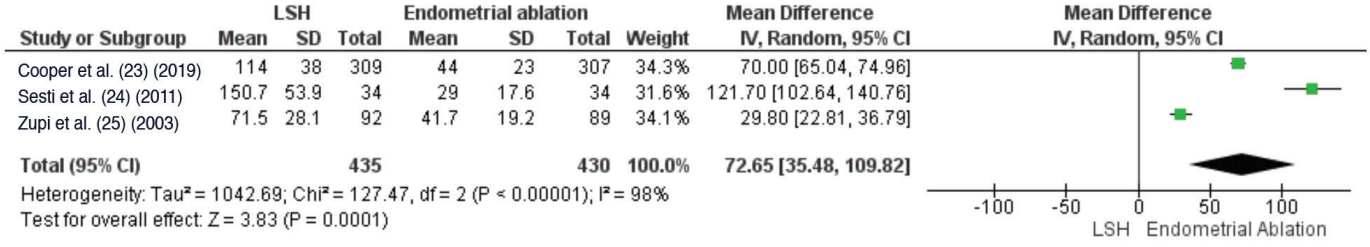

Figure 5. Forest plot for the analysis of operation time outcome
LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval

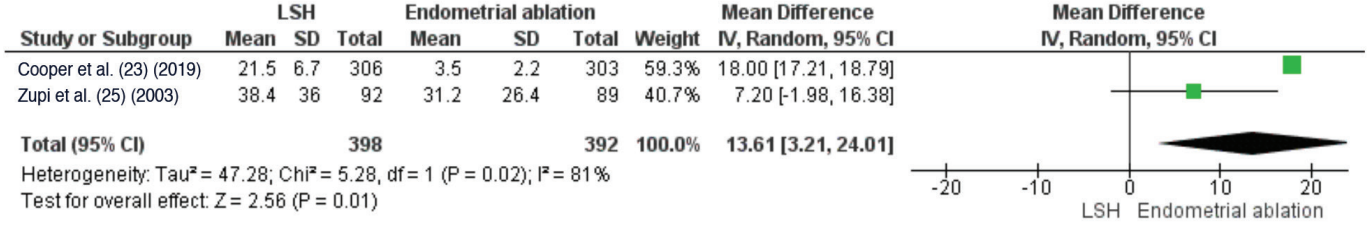

Figure 6. Forest plot for the analysis of time from operation to discharge outcome
LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval

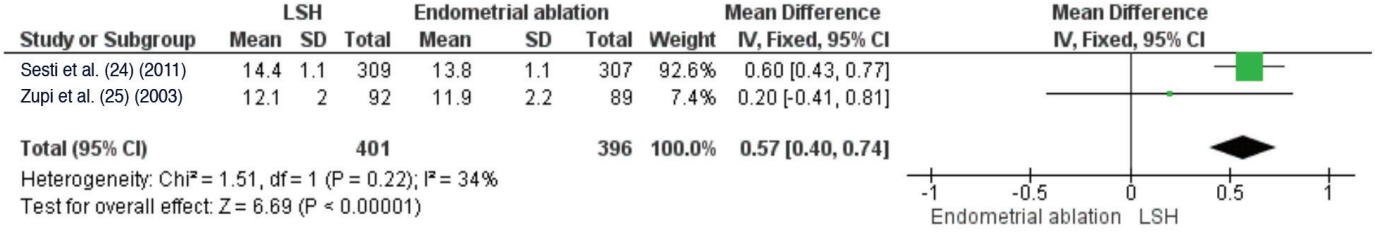

Figure 7. Forest plot for the analysis of hemoglobin outcome
LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval

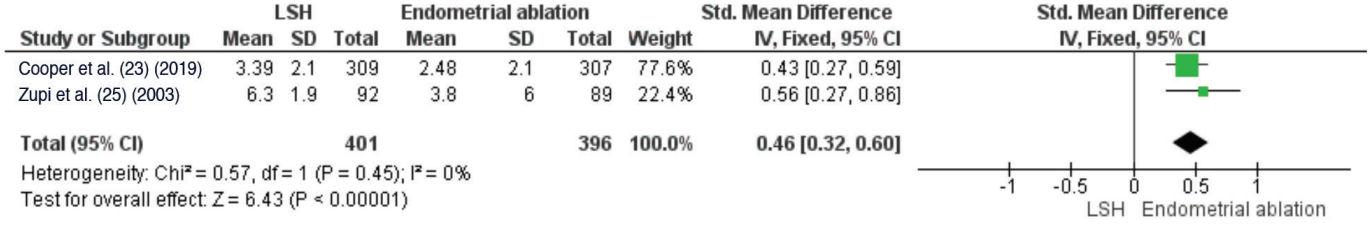

Figure 8. Forest plot for the analysis of pain outcome
LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval

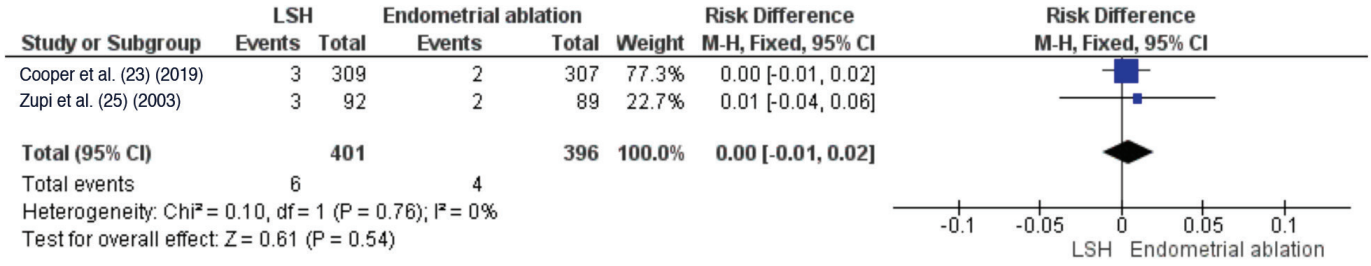

Figure 9. Forest plot for the analysis of fever outcome
LSH: Laparoscopic supracervical hysterectomy, SD: Standard deviation, CI: Confidence interval

show a significant difference between the groups in terms of quality of life, except for general health and social function, one study, Cooper et al. (30) reported in a later paper higher satisfaction and quality of life in the LSH group than the EA group at 15 months of randomization. In fact, this study showed there was only 3% dissatisfaction with LSH compared to a 13% dissatisfaction rate reported in the EA group. Moreover, 69% of women in the LSH group reported a maximum state of quality of life with a quality of life percentage score of 100, compared to only 55% of women who reported a 100% score in the EA group. Zupi et al. (31), 2003 showed similar results on their 2-year follow-up questionnaire. In contrast, O'Connor et al. (32), reported no significant statistical difference regarding satisfaction in the LSH group and the EA group. The follow-up study by Zupi et al. (33), 2015 also showed a better quality of life regarding physical and mental components after 14 years. In addition, Sesti et al. (34) reported a worsening of quality of life in the EA group between 12 months and two years. This is consistent with other non-randomized trials which reported improvement of psychological outcomes and quality of life after hysterectomy (35,36).

LSH was significantly better than EA regarding postoperative hemoglobin level, which usually reflects the overall amount of blood loss in each group in the three months following the operation. This was in spite of statistically significant longer operation time and a longer duration of hospital stay in the LSH group compared to the EA group. In a later analysis, Cooper et al. (30) showed similar results. The only reasonable explanation for this relative drop of hemoglobin level in the EA group, is that the intact uterus must in some cases still be producing cyclic menstrual bleeding despite the surgery, and that the LSH group was likely no longer menstruating in any reasonable capacity.

Other authors, however, have reported that TBA caused a significant decrease in menstrual bleeding and in some cases better hemoglobin levels postoperatively (37). Middleton et al. (38) reported that LSH had adverse impacts on the emotional state of patients. These authors explained their results as coming from the psychological effects of the patients losing their uterus in hysterectomy (38).

Conversely, EA showed significantly lower reported pain compared to LSH in our analysis. This may explain why many women prefer EA to LSH, as it is truly a less invasive procedure, and many women may be afraid of the rare, but possible, major complications that could occur during hysterectomy (39). However, no major complications were reported in our included studies of LSH. This result was also reported in a recent Cochrane study that failed to find any major complications in their reported groups (40). Therefore, we feel it is appropriate to stress that LSH is associated with better improvement in dyspareunia and cyclic pain with no higher risk of postoperative complications (30) than EA by several authors.

Study Limitations

The main limitations of our study are the paucity of clinical trials that compare the two surgical treatment procedures and the low sample size. Another limitation would be that our analysis was limited to those studies using specific forms in the assessment of the quality of life of the patients receiving the surgical procedures. This could explain, in addition to the different follow-up durations, the great heterogeneity in the analysis of most of our outcomes.

Despite these limitations, this is the first meta-analysis the authors are aware of that compares supracervical hysterectomy as a minimally invasive technique with EA or resection as a more conservative minimally invasive surgical procedure. As for the strong points of our analysis, all the studies included were clinical trials, which helped to ensure the most substantial evidence, as highlighted by the GRADE tool. Another strong point of our analysis was that all the included studies were found to be generally at a low risk of bias using our assessment tools.

Conclusion

LSH may have better outcomes in regards to quality of life than EA, specifically regarding patient reported social health, patient reported general health, and clinical postoperative hemoglobin

levels. EA, however, seems to have less operative time and a shorter hospital stay. Therefore, EA is also considered to be a reasonable technique with satisfying outcomes for many women suffering from AUB.

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Informed Consent: *Not applicable to Systematic Review.*

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Breast tuberculosis: a diagnosis not to be forgotten

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Abstract

Objective: To study the clinical characteristics and imaging features of breast tuberculosis (TB) and to describe treatment.

Material and Methods: A retrospective study including all patients hospitalized in the infectious diseases department for breast TB between 1997 and 2018.

Results: Twenty-two women, with a mean age of 39±12 years, were identified. In total, 18 patients were multiparous (81.8%). Both lump and mastalgia were the presenting symptoms in 19 cases (86.3%). Ipsilateral axillary lymphadenopathy was noted in 14 cases (63.6%). The most common finding on ultrasound was a well or poorly defined mass lesion, noted in 17 cases (77.2%), followed by fistulous tracts in seven cases (31.8%). Mammography showed focal, asymmetric breast density in 17 cases (89.5%) and diffuse in two cases (10.5%). The diagnosis was confirmed based on the presence of epithelioid cell granulomas and caseous necrosis in 13 cases (59.1%). Patients received antitubercular therapy for a mean duration of 11±5 months. The disease evolution was favorable in 20 cases (91%). There were two relapsing cases (9%).

Conclusion: Breast TB should be considered in the differential diagnosis of young patients presenting with palpable lump with axillary lymphadenopathy, especially in endemic regions. The diagnosis confirmation usually requires an excision biopsy providing histological or bacteriological evidence. (J Turk Ger Gynecol Assoc 2021; 22: 107-11)

Keywords: Breast, tuberculosis, ultrasonography, mammography, lymphadenopathy

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Introduction

Tuberculosis (TB) remains a public health issue, with an estimated 10 million new cases annually, worldwide (1). It is a multisystem disease that might involve any organ system. Breast TB is a rare disease, even in developing countries (2). Its incidence reaches 4% of all breast lesions in endemic regions (3). Patients usually consult for an isolated lump, and usually without constitutional symptoms of TB, which are present in around 20% of the cases (4). The diagnosis is often delayed due to the low index of suspicion and similarities with other breast pathologies. Breast TB is often mistaken for pyogenic abscesses or breast cancer (5). Imaging findings are often helpful in order to guide clinicians. However, breast TB has no specific ultrasonographic findings, which might present as a heterogeneous, hypoechoic, irregularly bordered mass with internal echoes or thick-walled cystic lesions (6).

Although Tunisia is a country with an intermediate rate of endemic TB (7), breast TB remains a very rare disease, representing 0.2% of extrapulmonary TB cases (8). In this context, the aim of this work was to study the clinical characteristics and imaging features of breast TB and to describe treatment.

Material and Methods

Study design

A retrospective study was conducted including all patients hospitalized in the infectious diseases department for breast TB over a 22-year period, between 1997 and 2018.

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Data collection and case definitions

Data was collected from the patients' medical records. The age, gender, place of residence (urban/rural) and previous medical history of patients were recorded. Clinical symptoms, laboratory investigations, microbiological and histopathological results, and imaging features were reviewed. The treatment received, its duration and the disease evolution were also collected.

Patients included in the study had microbiologically or histopathologically confirmed breast TB. By default, patients had clinical and radiological signs suggestive of the diagnosis, which were followed by an adequate response to antitubercular therapy.

According to the clinical presentation, fine needle aspiration or excision biopsy was performed in order to obtain a specimen for histological and microbiological (Ziehl-Neelsen staining) examination.

We haven't obtained ethical approval because of the non-interventional design of the study. Written informed consent was obtained from all patients.

Statistical analysis

Statistical analysis was performed using SPSS, version 20 (IBM Inc., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages. Continuous variables were presented as means and standard deviations, if they were normally distributed. Median and interquartile ranges were used for non-normally distributed data.

Results

Patient characteristics

During the study period, 22 patients were confirmed to have breast TB. All patients were females. The mean age was 39±12 years. Twelve patients were aged between 20 and 40 years. (54.6%) and 11 patients were from rural areas (50%). Three patients had a previous medical history of treated TB (13.6%). One patient had a family history of pulmonary TB (4.5%). At the time of diagnosis, five patients were pregnant (22.7%), and three patients were lactating (13.6%). In total, 18 patients were multiparous (81.8%) and four patients were nulliparous (18.2%) (Table 1).

Both lump and mastalgia were the presenting symptoms in 19 cases (86.3%). Ipsilateral axillary lymphadenopathy was noted in 14 cases (63.6%) and breast abscess in 11 cases (50%). Laterality was reported as unilateral in 21 cases (95.5%) and bilateral in one case (4.5%). The mean size of the lump was 5.1±2.8 cm. The lumps were located in the upper outer quadrant of the breast in 10 cases (45.5%). Associated with breast TB, other foci of TB were noted in four cases (18.2%), represented by cervical lymph node TB in all four (Table 1).

The median delay in seeking medical care was 40 (27-75) days. Elevated C-reactive protein level was noted in 10 cases (45.5%) with a median level of 42 (25-53) mg/L. An accelerated erythrocyte sedimentation rate was noted in seven cases (31.8%). Tuberculin skin test was positive in 13 cases (59.1%). All patients tested negative for human immunodeficiency virus.

Imaging features

Ultrasound was performed in all cases. The most common finding was a well or poorly defined mass lesion, noted in

Table 1. Characteristics of patients with breast tuberculosis

Variables	Number	Frequency (%)
Age categories (years)		
20-40	12	54.6
41-60	8	36.4
>60	2	9
Clinical symptoms		
Lump	19	86.3
Mastalgia	19	86.3
Ipsilateral axillary lymphadenopathy	14	63.6
Breast abscess	11	50
Breast fistula	7	31.8
Nipple discharge	5	22.7
Fever	8	36.4
Weight loss	3	13.6
Disseminated lesion	2	9
Skin ulceration	1	4.5
Location		
Right side	11	50
Left side	10	45.5
Bilateral	1	4.5
Asocciated site		
Lymph node	4	18.2
Pulmonary	2	9
Neuromeningeal	1	4.5
Diagnosis confirmation		
Histopathological proof: Epithelioid cell granulomas and caseous necrosis	13	59.1
Bacteriological proof: detection of *Mycobacterium tuberculosis**	4	18.2
Clinically confirmed	5	22.7
Disease evolution		
Favorable	20	91
Relapse	2	9
*: In microscopic smear or culture		

17 cases (77.2%). Fistulous tracts were noted in seven cases (31.8%), and ductal dilatation with echogenic component in six cases (27.2%). The disseminated form was noted in two cases (9%) with multiple collections mostly containing debris.

Mammography, performed in 19 cases (86.3%), showed focal, asymmetric breast density in 17 cases (89.5%) and diffuse in two cases (10.5%) (Figure 1). Skin thickening was noted in five cases (26.3%), multiple mass lesions in two cases (10.5%) and microcalcifications without suspect grouping in three cases (15.7%).

Magnetic resonance imaging was performed in three cases (13.6%). One patient presented with a bilobed mass enhancement with intermediate intense T2, surrounded by a hypointense, regular and fine halo on T1, and an early and heterogenous enhancement (curve type 2). Multiple, confluent mass-enhancement with irregular margins was noted in one case. One of these masses presented as a rim enhancement pattern (Figure 2). Non-mass enhancement was noted in one case.

Intervention and therapeutic procedure

Excision biopsy was performed in seven cases (31.8%). In order to exclude a possible breast cancer, lumpectomy was indicated in 10 cases (45.5%) and mastectomy in two cases (9%). The co-existence of breast TB and breast cancer was diagnosed in one patient, requiring mastectomy. The lack of response to antitubercular therapy associated with lesions involving the entire breast was the second indication for mastectomy in another patient. Drainage of the abscess was indicated in one case (4.5%).

The diagnosis was confirmed based on the presence of epithelioid cell granulomas and caseous necrosis in 13 cases (59.1%). The presence of epithelioid cell granulomas without caseous necrosis was noted in seven cases (31.8%). The detection of *Mycobacterium tuberculosis* in smear or culture confirmed the diagnosis in four cases (18.2%) (Table 1).

Patients received antitubercular therapy for a mean duration of 11±5 months. Side effects of antitubercular therapy were noted in 10 cases (45.5%), represented by the occurrence of gastrointestinal symptoms in 5 cases (22.7%), the increase in hepatic enzyme levels (alanine aminotransferase and aspartate aminotransferase) in 3 cases (13.6%) and skin reactions in 2 cases (9%). The disease evolution was favorable in 20 cases (91%). There were two relapsing cases (9%). The median length of hospital stay was 7 (4-13) days.

Discussion

The diagnosis of breast TB cannot only rely on imaging results, which may be non-specific. A high index of suspicion followed by clinical and radiological examination and appropriate

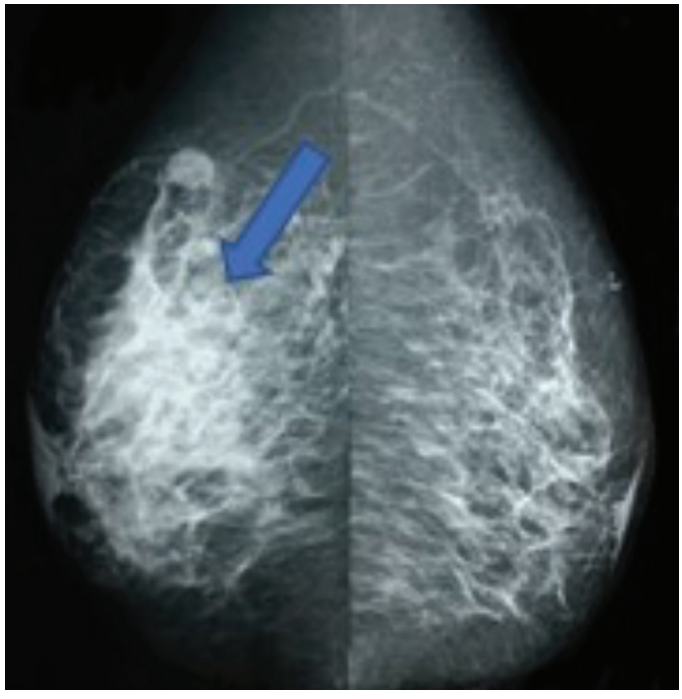

Figure 1. Standard craniocaudal view of the right and left breast shows an asymmetric density in the upper quadrant of the right breast (arrow)

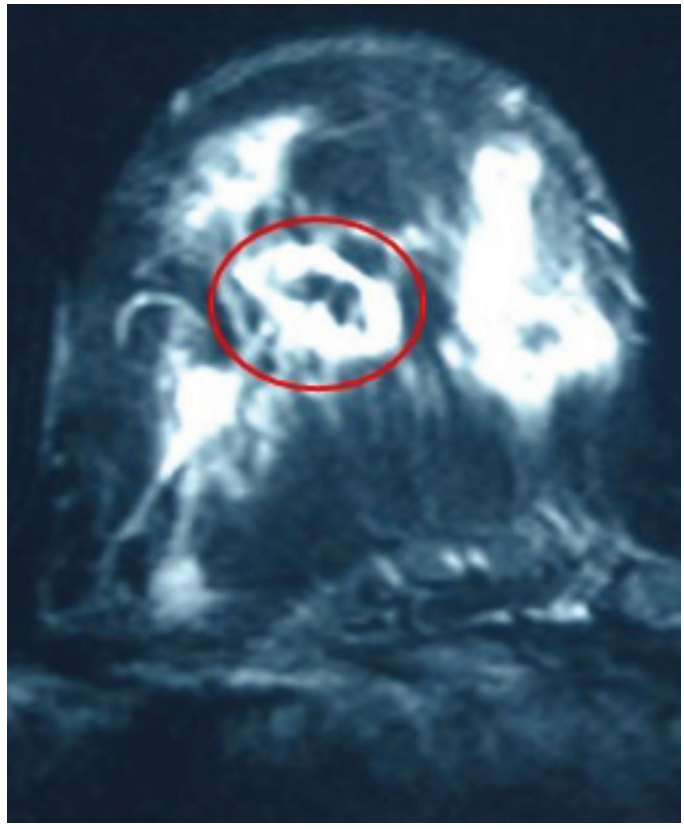

Figure 2. Breast magnetic resonance imaging shows multiple mass enhancement and a mass (encircled) with a rim enhancement

biopsies are mandatory in order to confirm the diagnosis of breast TB. It remains a rare disease affecting commonly young, multiparous and lactating women (9). Its rarity is explained by the resistance of breast tissue to the infection, therefore inhibiting the survival and multiplication of *Mycobacterium tuberculosis* (10). However, during lactation, the breast is highly vascularized, and the ducts are dilated which explains the susceptibility to TB infection (11). A previous study, including 65 cases of breast TB, reported a rate of 10.6% of lactating patients (8), which was similar to our results.

Breast TB was initially classified as primary when no other focus of TB was detectable, and secondary when it was associated with another focus of TB elsewhere in the body (12). Later, it was reported that breast TB is usually secondary, even if no other focus of TB was clinically or radiologically evident (13). We found other sites of TB associated with breast TB in 18.1% of the cases, while a previous study reported a rate of 67.9%, which was mostly represented by pleural and pulmonary TB (14). This discordance might be explained by the misdiagnosis of other sites of TB in our study.

In fact, secondary breast TB is commonly due to retrograde lymphatic spread from axillary lymph nodes, less commonly from cervical or mediastinal lymph nodes (15), which explains the frequent ipsilateral axillary lymphadenopathy associated with breast TB. Other sources of infection were reported, including hematogenous, or contiguous spread from the pleura or the chest wall (16).

Three types of breast TB have been reported, based on the clinical, radiological and pathological aspect of the disease. The nodular type is the most common, followed by the disseminated and the sclerosing form (17). Previous studies reported that the elderly present commonly with the nodular form, which mimics breast carcinoma clinically and radiologically (18,19), while younger patients usually present with pyogenic abscess (19,20).

Imaging features confirm the presence of the three forms of the disease, found during the clinical examination. The nodular form presents on mammography as an ill-defined density, which mimic breast cancer (21). The disseminated form is characterized by sinus formation secondary to multiple foci of TB (22). The irregularity of its margin might represent the tunneling of the abscesses and the associated blurring of the fat due to inflammatory reaction and edema (23), which mimic inflammatory carcinoma (15). The sclerosing form is seen when there is extensive fibrosis rather than caseation, and the entire breast hardens with a retracted nipple (4). Strong evidence of a tuberculous abscess is rarely found on mammography, which is represented by a dense sinus tract connecting an ill-defined breast mass to a localized skin thickening (24).

Imaging findings are of great interest in defining the extent of the lesion. However, distinguishing breast TB from breast cancer usually remains difficult (25). The simultaneous occurrence of breast cancer and TB has rarely been reported, and this diagnosis helps in down staging of carcinoma of breast and further management of the case (26). An excision biopsy, obtaining adequate tissue samples, is strongly recommended, which is not usually the case with fine needle aspiration (17). Demonstration of bacilli by microbiological, cytopathological or histopathological methods confirm the diagnosis of breast TB (2). This disease can present a diagnostic problem on radiological and microbiological investigations, and thus a high index of suspicion is needed.

The cornerstone of treatment remains antitubercular therapy prescribed for a minimum of six month (11). In order to prevent recurrence (27), or when the clinical response is slow (24), a prolonged treatment duration has been reported, as was the case with our patients. In fact, the associated site of TB may also interfere in the treatment duration, such as patient who presented with tuberculous meningitis. Along with medical therapy, surgical intervention might be required, if there is a lack of response to antitubercular therapy or due to the presence of lesions involving the entire breast (17). Another indication of mastectomy is the co-existence of breast TB and breast cancer, like the case of one of our patients. In fact, previous studies reported that radical mastectomy is indicated in the presence of clinically operable lesion, followed by antitubercular treatment, otherwise, palliative therapy combined with antitubercular treatment is indicated (28).

Conclusion

In our country, breast TB remains a rare and overlooked disease. The disease should be considered when young patients present with a palpable lump with axillary lymphadenopathy, especially in endemic regions. Although, imaging results were non-specific, they might aid in guiding clinicians. However, confirmation of a diagnosis of TB usually requires an excision biopsy providing histological or bacteriological evidence of infection.

Ethics Committee Approval: *We haven't obtained ethical approval because of the non-interventional design of the study.*

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

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F.H., M.K., A.C., M.B.J.; Data Collection or Processing: F.H., M.K., F.S., M.B.J.; Analysis or Interpretation: F.H., M.K., Y.H., C.M., M.B.J.; Literature Search: F.H., M.K., A.C., F.S., M.B.J.; Writing: F.H., M.K., M.B.J.

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Management of patients with urinary tract endometriosis by gynecologists

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Abstract

Objective: The aim was to report the postoperative outcomes of urinary tract endometriosis (UTE), which is a form of deep, infiltrative endometriosis, and to contribute to the literature by presenting our experience.

Material and Methods: In the present study, patients who underwent surgery for endometriosis at our clinic between 2005 and 2019 and had a final pathological diagnosis of UTE were examined in detail. Patient information was retrospectively retrieved from the medical records. Data obtained pre-, peri-, and postoperatively were analyzed.

Results: Mean age of the 70 patients included, according to the study criteria, was 32.73±7.09 years. Ureteral involvement alone was observed in 49% (n=34) of the patients, bladder involvement alone was observed in 24% (n=17) of the patients, and both bladder and ureteral involvement were observed in 27% (n=19) of the patients. Microscopic hematuria was detected in 16% (n=11) of the patients, whereas preoperative urinary tract findings, such as recurrent urinary tract infections, were detected in 19% patients (n=13). Of the patients, 56% (n=39) were identified with dyspareunia, 56% (n=39) with dysmenorrhea, and 30% (n=21) with pelvic pain. Visual analog scale score was significantly lower after the procedure ($p<0.0001$).

Conclusion: Although postoperative results were typically considered positive, surgical method performed in deep infiltrative endometriosis should aim to preserve fertility, improve quality of life, and reduce the complication rate to a minimum. (J Turk Ger Gynecol Assoc 2021; 22: 112-9)

Keywords: Urinary tract endometriosis, hematuria, dyspareunia, dysuria

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Introduction

Endometriosis is a disease characterized by the localization of functional endometrial tissue outside the uterus, which often affects the genital organs (1,2). Although it is most commonly reported among women in the reproductive age group, its exact prevalence is unknown. However, studies have shown that it is observed in the general population with a prevalence reported between 5% and 10% (3). The literature defines three types of endometriosis-superficial endometriosis, deep infiltrative endometriosis (DIE), and ovarian endometrioma (4). DIE is an aggressive form and is defined as an invasion of the peritoneum deeper than 5 mm (5). The intestines, urinary tract, uterosacral ligament, and vagina are often affected. The symptoms may become more apparent with increase in the depth of infiltration (6-8).

Although the reported prevalence of urinary tract endometriosis (UTE) is 1%-6%, this includes bladder and ureteral involvement, which is an aggressive form of DIE (6,9). UTE is observed in 14%-20% of all patients with DIE (10). The bladder and ureter, which are the most frequently involved sites, are

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affected at ratios of 84% and 10%, respectively (11-13). In UTE, which may be challenging to diagnose, endometriosis with bladder involvement should be considered if the patient describes frequent urination, cyclical bladder pain, dysuria, or hematuria (14). Endometriosis with ureteral involvement is typically asymptomatic and unaccompanied by genitourinary symptoms in 50% of patients. It is observed together with back and pelvic pain (9,15,16). Magnetic resonance imaging (MRI) and ultrasonography (USG) exhibit a high specificity for endometriosis with bladder involvement in the diagnosis of UTE in which symptoms are highly non-specific. However, both techniques are inadequate in identifying lesions <3 cm (17). Evidence concerning the diagnosis of endometriosis with ureteral involvement is extremely limited (2).

Laparotomy or laparoscopy can be preferred for surgical treatment of UTE in cases in which extremely limited medical approaches are available. However, laparotomy is not preferred due to the inability to completely view lesions, as well as lengthy hospitalization and greater blood loss. Nevertheless, despite the disadvantages of laparotomy, laparoscopy is typically not performed in the treatment of DIE (2).

The aim of this study was to present the treatment of patients with DIE characterized by bladder and ureteral involvement and its long-term outcomes. It was hoped that by presenting our experience in terms of effectiveness, safety, and complication rates of the most suitable approaches for the patient with UTE, characterized by bladder and ureter involvement, management of this challenging condition could be made somewhat simpler.

Material and Methods

This was a retrospective study, which was performed at the obstetrics and gynecology clinics of a university hospital between 2005 and 2019. All aspects of this research were approved by Ethics Committee of Atatürk University of Medical Sciences.

In this study patients who underwent surgery for endometriosis in our clinic between 2005 and 2019 and had a final pathological diagnosis of UTE were examined in detail. The pathological, clinical, and medical records were reviewed, and surgery type, complication rates, preoperative complaints, and duration of hospital stay were recorded. Patients with rectovaginal involvement, deep pelvic invasion, and complete or partial obliteration of the pouch of Douglas, together with an ovarian endometrioma were considered to have advanced stage (stage 3/4) disease according to the American Fertility Association scoring system (18). Based on their surgery records, patients with bladder and ureteral involvement who underwent ureterolysis, had partial or complete resection of the urethra, had bladder nodule resection, or had partial bladder resection were selected for detailed analysis.

From the medical records, it was determined whether the preoperative clinical evaluation of the patients was performed by a multidisciplinary team and whether the operating team was decided by establishing an expert committee, as well as the surgical approach used and the necessity of the imaging method used. All patients had undergone preoperative urinary USG, transvaginal USG, and renal function tests.

In cases with large ureteral nodules, the nodule was removed via the incision of the ureter and end-to-end anastomosis was performed. The bladder was sutured twice (Vicryl 3/0 or Monocryl 3/0) in cases wherein the bladder was completely opened. At the end of the procedure, 200-300 mL of 0.9% NaCl solution diluted with indigo carmine was injected into the bladder for a fluid leakage test. Prophylactic antibiotics were administered to all patients preoperatively.

Postoperative follow-ups were performed at Atatürk University multidisciplinary endometriosis center-the tertiary referral center of the region-at the end of the first and sixth months. The management of patients who developed complications or who required recurrent surgery was performed by a multidisciplinary team. Preoperative and postoperative pain scores using a visual analog scale (VAS score) were obtained from medical records.

Statistical analysis

The analyses were performed using the SPSS, version 20 (IBM Inc., Armonk, NY, USA). Data are presented as mean, standard deviation, median, minimum, maximum, percentage, and number. Normal distribution of continuous variables was evaluated using the Shapiro-Wilk W test when the sample size was <50 and using the Kolmogorov-Smirnov test when it was >50. In the comparison of continuous variables with more than two independent groups, ANOVA test was used when distribution was normal, whereas the Kruskal-Wallis test was used in cases where it was not met. In 2x2 comparisons between categorical variables, the Pearson's chi-square test was used when the expected value was >5, Yates' chi-square test was used when the expected value was between 3 and 5, and Fisher's exact test was used when the expected value was <3. In comparisons greater than 2x2 between categorical variables, the Pearson's chi-square test was used when the expected value was >5 and whereas the Fisher-Freeman-Halton test was used when the expected value was <5. The level of statistical significance level was set as $p<0.05$.

Results

Seventy patients were identified who met the study inclusion criteria. The mean age of the patients was 32.73±7.09 years. The surgical procedure was started laparoscopically in all patients. However, due to technical difficulties, laparotomy was initiated in

three patients. Ureteral involvement alone was observed in 76% (n=53) of the patients, bladder involvement alone was observed in 51% (n=36) of the patients, and both bladder and ureteral involvement was observed in 27% (n=19) of the patients.

Records showed that 29 (41%) patients received medical treatment within the six months prior to surgery. Twenty nine of 70 patients had been prescribed 2 mg of dienogest daily for 6 months as a medical treatment. Moreover, 16 (23%) patients in the study group had undergone previous pelvic surgery (Table 1). During the preoperative period, 12 patients suspected of DIE received double J stents. However, two of these patients had ureteral injury despite this and underwent end-to-end anastomosis. Hydronephrosis was preoperatively diagnosed using preoperative imaging in eight patients with ureteral involvement. It was found that bilateral double J stent was placed by urologists prior to surgery in patients suspected of having severe ureteral involvement. In cases preoperatively suspected of having a bladder nodule, cystoscopy was performed to determine the localization of the nodule at the beginning of the process and to identify whether the ureteral orifices, and trigone in particular, were involved. Although it was not possible to obtain the intrinsic and extrinsic distinction of ureteral involvement from medical records, it was observed that this was evaluated in the final pathological assessment of the patient. It was determined that patients with severe ureteral involvement together with symptoms initially underwent ureterolysis, whereas those who developed severe fibrosis and irreversible stenosis underwent resection. In endometriosis with bladder involvement, it was determined that depending on the size of the nodule and infiltration, either the bladder was completely opened or partially resected or the nodule was excised. The medical records revealed that urologists were included in the surgery if deemed necessary by surgeons experienced in endometriosis. Moreover, the excision of bladder nodules and extrinsic ureteral nodules was mainly performed by gynecologists, although in cases with more severe complications, specialist urologists were included in the surgery. The bladder catheter remained in place for an average of 10 days, whereas the ureteral catheters remained for 4 to 6 weeks.

Microscopic hematuria was detected in 16% (n=11) of the patients, whereas preoperative urinary system findings, such as recurrent urinary tract infections, were detected in 19% (n=13) of the patients. Of the patients, 56% (n=39) were identified with dyspareunia, 56% (n=39) with dysmenorrhea, 16 with cyclical recurrent dysuria, and 30% (n=21) with pelvic pain. No significant difference was observed between the groups regarding CA-125 levels. Postoperative pain scores using a VAS was significantly lower after the procedure.

Examination of the operative notes of the patients revealed that ureterolysis was performed in 27 (39%) patients, end-to-end anastomosis was performed in 11 (16%) patients, and no kidney loss was observed in any patient. During the follow-up period, four patients developed fistulas within 6 months; three of them had vesicovaginal fistula and one of them had rectovaginal fistula. Overall, five (7.1%) patients in the study group were re-operated in our clinic within a maximum of 6 months postoperatively (Table 2). No significant difference was observed among the three study groups (ureteral, bladder, ureteral and bladder) in terms of their preoperative

Table 1. Distribution of endometriotic nodules and patient symptoms

		n (%)
Ureteral nodule	No	17 (0.24)
	Yes	53 (0.76)
Bladder nodule	No	34 (0.49)
	Yes	36 (0.51)
Both ureteral and bladder nodule		**19 (0.27)**
Microscopic hematuria	No	59 (0.84)
	Yes	11 (0.16)
Recurrent urinary tract infection	No	57 (0.81)
	Yes	13 (0.19)
Dyspareunia	No	31 (0.44)
	Yes	39 (0.56)
Dysmenorrhea	No	31 (0.44)
	Yes	39 (0.56)
Dysuria	No	54 (0.77)
	Yes	16 (0.23)
Pelvic pain	No	49 (0.7)
	Yes	21 (0.3)
Preoperative medical treatment	No	41 (0.59)
	Yes	29 (0.41)
Previous pelvic surgery	No	54 (0.77)
	Yes	16 (0.23)

Table 2. Distribution of urogynecological complications and surgical procedures employed

		n (%)
Ureterolysis	No	43 (0.61)
	Yes	27 (0.39)
End-to-end anastomosis	No	59 (0.84)
	Yes	11 (0.16)
Ureterocystostomy	No	70 (0)
Fistula (detected within 6 months)	No	66 (0.94)
	Yes	4 (0.06)
Re-operation within a maximum of 6 months	No	65 (0.93)
	Yes	5 (0.07)

symptoms, urogram results, the medical treatment received for endometriosis preoperatively, and previous pelvic surgery (Table 3). When intra- and postoperative complications were examined, ureterolysis was significantly higher in the group with ureteral involvement. Ureterolysis was statistically significantly higher in both ureteral and bladder nodule group than bladder nodule group (p=0.016; Table 4). The upper limit for the CA-125 level was considered 35 IU/mL. When the groups were examined with respect to their CA-125 levels, the mean concentrations were 113±165 IU/mL in the group with ureteral involvement, 117±232 IU/mL in the group with bladder involvement, and 94±56 IU/mL in the group with both ureteral and bladder involvement which was not significantly different (Table 5). Furthermore, nine patients presented to the in vitro fertilization unit of our clinic within six months and that seven of them became pregnant.

The patients were questioned in detail especially when they were called for follow-up in terms of preoperative symptoms. Upon comparing the severity of symptoms of the patients at the end of the sixth postoperative month, the severity was reduced at least by half in 75% of the patients. When the postoperative pain scores using a VAS score were compared

Table 3. Comparison of symptoms according to endometriotic nodule localization

		Groups by nodule localization						
		Ureteral nodule		Bladder nodule		Both ureteral and bladder nodule		p
		Count	Column (n, %)	Count	Column (n, %)	Count	Column (n, %)	
Microscopic hematuria	No	26	0.76	16	0.94	17	0.89	0.250
	Yes	8	0.24	1	0.06	2	0.11	
Recurrent urinary tract infection	No	26	0.76	16	0.94	15	0.79	0.321
	Yes	8	0.24	1	0.06	4	0.21	
Dyspareunia	No	18	0.53	4	0.24	9	0.47	0.130
	Yes	16	0.47	13	0.76	10	0.53	
Dysmenorrhea	No	14	0.41	7	0.41	10	0.53	0.692
	Yes	20	0.59	10	0.59	9	0.47	
Dysuria	No	24	0.71	16	0.94	14	0.74	0.155
	Yes	10	0.29	1	0.06	5	0.26	
Pelvic pain	No	21	0.62	13	0.76	15	0.79	0.339
	Yes	13	0.38	4	0.24	4	0.21	
Preoperative medical treatment	No	20	0.59	12	0.71	9	0.47	0.369
	Yes	14	0.41	5	0.29	10	0.53	
Previous pelvic surgery	No	25	0.74	13	0.76	16	0.84	0.707
	Yes	9	0.26	4	0.24	3	0.16	

Table 4. Comparison between groups in terms of the complications of treatment approaches

		Groups according to nodule localization						
		Ureteral nodule		Bladder nodule		Both ureteral and bladder nodule		p
		Count	Column (n, %)	Count	Column (n, %)	Count	Column (n, %)	
Ureterolysis	No	20	0.59	15	0.88	8	0.42	0.016*
	Yes	14	0.41	2	0.12	11	0.58	
End-to-end anastomosis	No	29	0.85	15	0.88	15	0.79	0.761
	Yes	5	0.15	2	0.12	4	0.21	
Urogynecological fistulas	No	33	0.97	16	0.94	17	0.89	0.567
	Yes	1	0.03	1	0.06	2	0.11	
Re-operation within a maximum of 6 months	No	30	0.88	16	0.94	19	1.00	0.296
	Yes	4	0.12	1	0.06	0	0.00	
*Ureterolysis was statistically significantly higher in both ureteral and bladder nodule group than bladder nodule group								

with preoperative scores, the mean pain score was 5 before the procedure and this had reduced to 2 in the sixth month after surgical or medical treatment. VAS score was significantly lower after the procedure ($p<0.0001$) (Table 6). According to our medical records, patients did not die in the long term and did not apply to our urology or obstetrics and gynecology clinic with the same symptoms.

Discussion

Several studies in the literature have shown that DIE surgery, which has a high probability of complications, requires a multidisciplinary approach both in the pre- and postoperative periods and that it should be performed by an experienced team specialized in the field (19,20). Our study demonstrated that a multidisciplinary approach ensures an improvement in the quality of life and a significant reduction in pain. Postoperative pain scores using a VAS score was significantly lower after the procedure. With regard to this disease, for which rather different views have been reported in terms of treatment approaches, it is crucial that the surgical approaches employed for preventing recurrence and ensuring patient comfort primarily include the complete excision of nodules (19). In our study, 41% of patients had previously received medical treatment that was ineffective and that complete excision of the nodules was effective. Our higher rate of UTE, which is higher compared with that reported in the literature, can be attributable to the fact that our center is the largest endometriosis clinic in the region and that most of the patients were diffuse, advanced, and serious cases at the time of diagnosis (21,22). The operative notes of the patients who developed fistula showed that harmonic scalpel was used during dissection. Because the heat effect generated in the surrounding tissues by the energy can lead to thermal damage in the lateral tissues with the increase in operation time and temperature, it was considered as a factor in the occurrence of the damage.

Endometriosis with bladder involvement, which is observed in approximately 12% of patients with DIE, is characterized by a partial or complete involvement of the detrusor muscle layers (20,21). This may be owing to the fact that the detrusor muscle is stronger than ureteral muscles and has a denser vascular network that contributes to the healing process (22). The incidence of bladder involvement, which is a rare form of DIE, reported in the literature has gradually increased (19). It has been suggested that this may have an iatrogenic origin and the increased prevalence of endometriosis with bladder involvement might be particularly due to the increased rate of cesarean surgeries. It has been shown that 50% of endometriosis with bladder involvement occurs in women who have undergone a previous surgery (23). In our case series, 23% of the patients with UTE had undergone previous pelvic surgery. Four of our patients with bladder involvement had previously undergone pelvic surgery.

Studies have shown that the distal ureter is more frequently involved due to its proximity to the posterior compartment of the pelvic organs (24). Therefore, endometriosis, the pathophysiology of which remains unclear, has been associated with retrograde menstruation (2), and it is thought to be caused by the accumulation of menstrual blood and endometrial cells in the pelvis due to gravity and their presence at that location for a prolonged time (25). Based on this theory, it has been shown that rectovaginal involvement is more common than bladder involvement (26). Although 90% of women have retrograde menstruation, the reason for 10% of them developing endometriosis remains unclear. However, the effect of gravity, more frequent occurrence of endometriosis on the left side, and rarer occurrence of endometriosis with bladder involvement in the retroverted uterus, as well as the fact that menstrual blood remains more on the left side due to the sigmoid colon may provide an explanation for this theory remaining the most likely (2,20). In the study of Schonman et al. (19) half of the patients with endometriosis with bladder involvement had an ovarian

Table 5. Relationship between location of endometriotic nodules in the urogenital region and CA-125

		CA-125			
		Mean ± SD	**Median (min-max)**	**Chi-square**	**p**
Groups according to nodule location	Ureteral nodule	113±165	78 (7-961)	3.635	0.162
	Bladder nodule	177±232	120 (10-996)		
	Both ureteral and bladder nodule	94±56	78 (19-197)		
SD: Standard deviation, min: Minimum, max: Maximum					

Table 6. Comparison of VAS scores before and six months after treatment

Variable	**Before treatment (n=70) Median (min-max)**	**Six months after treatment (n=70) Median (min-max)**	**p**
Postoperative pain score (VAS)	5 (4-10)	2 (3-5)	0.033
VAS: Visual analog scale, min: minimum, max: maximum			

endometrioma and almost one-third of them had rectosigmoid lesions. Kovoor et al. (21) reported that 76% of patients with DIE were associated with deep endometriotic lesions in the pelvis, including rectovaginal, parametrial, and uterosacral nodules. Moreover, we found that ureteral involvement was higher than bladder involvement in UTE.

Endometriosis with ureteral involvement is a component of DIE. It is often non-symptomatic and complaints of the patients are non-specific. Patients typically describe complaints related to lateral pain and cystitis (22). Reportedly, 30% of patients remained asymptomatic and the diagnosis was incidental (27). At the time of diagnosis, decreased renal function can be observed in approximately one-third (30%) of patients and kidney loss may be present in 25%-43% patients (28). In the study conducted by Cavaco-Gomes et al. (29) on 700 patients with endometriosis with ureteral involvement, they reported no urinary system symptoms. They also found that 81% of patients described dysmenorrhea, 70% described pelvic pain, and 66% described dyspareunia (29). Schonman et al. (19) showed in their case series that 40% of their patients had urinary complaints. In our case series, 39 (56%) patients presented with complaints of dyspareunia, 39 (56%) patients with complaints of dysmenorrhea, and 21 (30%) with complaints of pelvic pain. In addition, one (16%) patient had microscopic hematuria and 13 (19%) patients had urinary tract symptoms, such as recurrent urinary tract infection. No renal loss was detected in any patient.

In the treatment of endometriosis, whether an aggressive surgical procedure or conservative approach will be preferred may vary depending on the preoperative evaluation of the patient, the experience of the surgeon, and the severity of the symptoms. Preoperative diagnosis is extremely controversial due to variations in the scope of lesions. USG and MRI have high specificity in detecting endometriosis with bladder involvement, but both are insufficient in identifying lesions <3 cm (17). Nezhat et al. (2) reported that they initially plan cystoscopy simultaneously with USG and laparoscopy prior to surgery when they suspect endometriosis with bladder involvement. Various studies have demonstrated that pelvic MRI achieves a sensitivity of up to 88% and specificity of up to 99% in the diagnosis of endometriosis with bladder involvement and that it has a diagnostic accuracy of approximately 98% (27). In our study, preoperative imaging methods were used depending on the severity of the disease and that USG and concurrent cystoscopy were performed in patients suspected of severe UTE. MRI was performed preoperatively in 80% of patients with UTE.

The primary clinical goals are to recover the damaged ureter, prevent kidney loss, and reduce patient complaints. Seracchioli et al. (30) successfully performed laparoscopic ureterolysis in 22 of 30 patients with endometriosis with ureteral involvement and treated eight other patients by performing ureteroureterostomy or ureteroneocystostomy. However, despite all these interventions, unpredictable complications, such as fistula, leakage after end-to-end anastomosis, and leakage after reimplantation, can be postoperatively observed (22). In their study, Rozsnyai et al. (31) reported complications in four patients treated for endometriosis with ureteral involvement and in two patients treated for endometriosis with bladder involvement. These complications were ureteric fistula due to thermal damage caused by harmonic scalpel and pyelonephritis, bladder atony, and vesicovaginal fistula following ureteroneocystostomy (31). During the postoperative period, recurrent surgery was necessary in five patients, vesicovaginal fistula in three patients, and rectovaginal fistula in one patient within 6 months.

Currently, the treatment of endometriosis with bladder involvement remains a controversial subject. Treatment may depend on various factors including the patient age, fertility status, disease prevalence, and symptom severity as well as presence of other pelvic lesions. Medical treatment may be preferred, depending on disease severity, but the high recurrence rates and symptom recurrence upon treatment discontinuation must be considered (19). However, as endometriosis is a progressive disease, following the surgical excision of endometriotic nodules, hormonal treatment can be administered to prevent recurrence and reduce pain (32).

In their study, Fedele et al. (33) demonstrated that even if medically induced amenorrhea is achieved, endometriotic nodules regress by only 30%. Smooth muscle fibers and fibrosis constitute 60% of the histopathological component of the nodule that does not respond to hormonal treatment, whereas the remaining tissue is the endometrial epithelium (34). Price et al. (23) argued for both medical and surgical approaches in treatment, but observed surgical excision to be more effective. In our study, a significant portion of the patients had previously received hormonal treatment, and the surgical treatment approach had become inevitable thereafter. We believe that a laparoscopic approach performed by experienced surgeons is the preferred treatment method for both ureteral and bladder involvement in severe cases of endometriosis, and our study shows that long-term outcomes are extremely satisfactory.

Conclusion

The surgical method used in DIE, the pathophysiology of which is not understood and the optimal treatment methods remain unclear, should aim to preserve fertility, improve the quality of life, and reduce the complication rate to a minimum. We also recommend performing abdominal ultrasound in all DIE patients prior to surgery. This study

has shown that the long-term outcomes of this challenging surgical procedure performed by experienced surgeons are extremely satisfactory.

Ethics Committee Approval: *All aspects of this research were approved by Ethics Committee of Atatürk University of Medical Sciences.*

Informed Consent: *All participants had completed informed consent before surgery.*

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Comparison of the rates for reaching the blastocyst stage between normal and abnormal pronucleus embryos monitored by a time-lapse system in IVF patients

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Abstract

Objective: To compare the rates of blastocyst stage development between embryos fertilized after one (MPN) or more than two pronucleus (PN) (3PN, 4PN-multiPN) with those after 2PN in the same patients.

Material and Methods: The embryos of patients who had both abnormal PN (MPN, 3PN or 4PN) and normal fertilized (2PN) embryos after intracytoplasmic sperm injection (ICSI) fertilization, were followed with a time-lapse system following the ICSI procedure. The rates of reaching the blastocyst stage were compared between normal and abnormally fertilized embryos.

Results: One thousand eight hundred and twenty oocytes were collected from 140 patients and 1280 (70.3%) of them were fertilized. MPN, 2PN and 3PN, 4PN (multiPN) ratios of the embryos in the pronuclear stage were 11.4%, 83.13% and 5.47%, respectively. The rates of reaching the blastocyst stage among these embryos were 17.1%, 60.8% and 42.8% for MPN, 2PN and multiPN, respectively. The proportion reaching blastocyst development was significantly higher following 2PN compared to those after MPN and multiPN ($p<0.05$). Embryos developing after multiPN had significantly higher rates of reaching the blastocyst stage compared to those after MPN ($p<0.01$).

Conclusion: The majority of abnormally pronucleated embryos arrest without reaching the blastocyst stage. MultiPN embryos have a higher rate of blastocyst development than MPN embryos. (J Turk Ger Gynecol Assoc 2021; 22: 120-6)

Keywords: Blastocyst, ICSI, MPN, multiPN, time-lapse

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Introduction

Successful fertilization is the first parameter that is checked following the combination of oocyte and sperm in assisted reproduction treatments. Two pronucleus (PN) and two polar bodies (PB) are observed 16-18 hours after fertilization under normal conditions. However, an abnormal number of pronuclei (1, 3 or 4) can be seen if abnormal fertilization has occurred.

The reason for the presence of only one pronucleus (MPN) in the embryo after fertilization has been investigated by many researchers (1-10). MPN and one or two PB's are observed in 2.7-17% of embryos after in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedures (1). Embryos with MPN usually have two PB's (1). Plachot (2) reported that the incidence of MPN was around 1% in IVF or ICSI cycles.

The zygotes with MPN were thought to be due to parthenogenetic activation rather than spermatozoon fertilization (3). However,

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sperm penetration findings were observed in approximately 45% of MPN zygotes, providing evidence for their development after fertilization (4). The presence of Y chromosomes in the genetic structure of MPN zygotes with preimplantation genetic diagnosis was reported as a proof of fertilization (5). Most embryos that develop from MPN have been shown to be aneuploid, even though they contain Y chromosomes (6).

The presence of MPN may be due to errors in the formation or fusion of the pronucleus during fertilization (7). Failure of male or female chromatids to form a pronucleus causes MPN. Flaherty et al. (8) observed swollen sperm head (52%), intact, undecondensed sperm head (28%), and ejection of the spermatozoon (20%) in one-pronuclear oocytes. The formation of MPN embryos was also based on the asynchronous occurrence of pronucleus, the union of male and female pronucleus, and male or female parthenogenesis (9). The asynchronous view of pronucleus formation has been assessed as the most likely explanation for the presence of biparental diplodia in embryos developing from MPN and some of these embryos were reported to be transferable (10).

The presence of more than two pronuclei in the fertilization control stage, rather than the expected 2PN, is called multiPN. The rate of 3PN formation was 1% and 5% after ICSI and IVF procedures, respectively (11). Feenan and Herbert (12) considered the presence of more than two pronuclei to be associated with genetic disorders. They concluded that out of 3PN embryos, 61.8% had a triploid chromosome component, 25.2% had mosaic sequencing, and only 12.6% carried a diploid chromosome set (12). More than two PN formation may be triggered by inability to extrude the second polar body, incomplete chromatid separation to the extruded polar body, and dispersion of oocyte chromatids in the second polar body formation (13).

Early cleavage may appear normal in 3PN zygotes, but progression may cease or aneuploidy may occur later (14). After IVF, 3PN zygotes may develop into different embryo stages with variable chromosomal component (triploidy; XXY, XXX, XYY, diploidy, mosaic) (15).

A time-lapse system allows observation of embryonic development in detail and provides algorithms to select high quality embryos according to the kinetic and morphologic changes. It is important to select embryos with high implantation potential for transfer in assisted reproduction. Abnormally fertilized oocytes, depending on how they are formed, either develop to different stages or arrest, usually from the first day. Those which develop to the fifth day are not preferred for transfer because they have low scores. The aim of this study was to compare the rates of reaching the blastocyst stage among abnormally fertilized embryos developing after MPN and multiPN with those of the normally fertilized embryos after 2PN using a time-lapse system.

Material and Methods

Study design

This was a retrospective study conducted on IVF patients treated at an IVF center, between January 2013 and December 2017. The study protocol was approved by the Institutional Review Board of the Maltepe University Faculty of Medicine (approval number: 78, date: 22.09.2017). The study included 140 patients. Consent forms were obtained from the patients. Inclusion criteria were patients aged 25-40 years, patients who had both abnormal PN (MPN, 3PN or 4PN) and normal fertilized (2PN) embryos after fertilization, with all embryos followed with a time-lapse system following an ICSI procedure. The development of fertilized oocytes from the first day to the fifth day was examined. The rates of reaching the blastocyst stage between normal and abnormally fertilized embryos were compared.

Ovarian stimulation

The standard ovarian stimulation protocol of our clinic has been reported previously (16). Pituitary down-regulation was performed either by the gonadotropin-releasing hormone agonist (GnRHa) leuprolide acetate (lucrin: 0.5 mg/mL, Abbott, Spain) or GnRH antagonist, cetrorelix acetate (Cetrotide, Baxter Oncology GmbH, Germany). Leuprolide acetate was used daily in the late luteal phase before the treatment cycle and cetrorelix acetate was started daily on the 5th day of the treatment and was continued until ovulation was triggered. Gonadotropin injections were started on cycle days 2 or 3, if >2 cm cysts were not observed by baseline ultrasound in patients. The daily dosage for gonadotropin stimulation was individualized between 150 and 300 IU. Patients were monitored by ultrasound until the trigger criteria, including three follicles with maximum diameter >17 mm, were present. Human chorionic gonadotropin (hCG) 10000 U (Choriomon, IBSA, Italy) and 5000 U hCG (Choriomon, IBSA, Italy) plus 0.2 mg triptorelin acetate (Gonapeptyl, Ferring GmbH Liel, Germany) were used for oocyte maturation in the agonist cycles and the antagonist cycles, respectively. Patients underwent transvaginal ultrasound-guided oocyte retrieval with a 17-gauge needle, under general anesthesia, 35-36 hours after hCG administration. The oocyte-corona complexes were denuded, incubated for 2 hours and ICSI was performed. ICSI is routinely performed in all patients as our clinical policy.

Preparing EmbryoScope dish

Micro-wells of the EmbryoScope dish were filled with pre-warmed culture solution. Wells were filled with ~25 μl solution without any bubbles and were covered with 1.4 mL ovoil. The

dishes were incubated at 37 °C. Each injected oocyte was placed in a well without any bubbles and slides were placed into the time-lapse system (EmbryoScope, Vitrolife, Sweden).

Embryo selection

Patient information and the quality of embryos observed were recorded every day by the EmbryoScope. The main issue for selection of an embryo for transfer was to be at an appropriate stage according to post-insemination time. Size and number of PNs were considered. Size and evenness of the blastomere, fragmentation (>10%), multinucleation, vacuole (>14 μm), and cleavage times were evaluated at the early cleavage stages. Cell numbers that join the cell compaction were considered for the next stage, and then, inner cell mass and trophectoderm were notated for the blastocyst stage embryo.

Statistical analysis

All analyses were carried out by Number Cruncher Statistical System 2007 (Kaysville, Utah, USA). Univariate and mulivariate Generalized Linear Mixed models were used to examine the effect of PN number on blastocyst development and the effect of age and etiologies of infertility on PN number. A $p<0.05$ was considered to indicate statistical significance.

Results

Demographic and clinical characteristics of the patients are summarized in Table 1.

Table 1. Demographic and clinical characteristics of the patients

(n=146)		
Age (years)	Range	25-40
	Mean and standard deviation	32.74±4.32
BMI	Range	16.2-41.5
	Mean and standard deviation	23.48±4.48
Indications		**n (%)**
	Unexplained	46 (31.5)
	Male	46 (31.5)
	Anovulation	14 (9.6)
	Tubal factor	23 (15.8)
	Endometriosis	15 (10.3)
	Poor ovarian reserve	16 (11.0)
Number of previous embryo transfer	0	37 (25.2)
	1	9 (6.2)
	2	96 (65.8)
	3	2 (1.4)
	4	2 (1.4)
BMI: Body mass index		

The number of oocytes collected from the 140 patients in the study was 1820. Fertilization was observed in 70.3% (n=1280) of the oocytes (Table 2). Among the fertilized oocytes, 146 were MPN (11.4%), 1064 were 2PN (83.1%) and 70 were multiPN (5.5%) (Table 2). Abnormal fertilization (MPN, multiPN) was observed in 216 embryos with an abnormal fertilization rate of 16.9%. Out of 1,280 fertilized embryos, 702 (54.8%) reached the blastocyst stage.

The embryos were divided into three groups, MPN, 2PN and multiPN, according to the number of pronuclei observed on the first day and the rates of blastocyst development were calculated for each group. Blastocyst development was observed in 17.1%, 60.8% and 42.9% of the embryos developing after MPN, 2PN and multiPN, respectively (Table 3).

A generalized linear mixed models analysis was used to examine the effect of PN number on blastocyst development. The model obtained was statistically significant, thus the number of PN had a statistically significant effect on the success of blastocyst development ($F=43,731$; $p<0.001$) (Table 4). When binary evaluations were examined, blastocyst development percentage was higher in the 2PN oocytes than MPN and multiPN groups ($p<0.001$; $p=0.015$, respectively). When abnormally fertilized groups were analyzed, blastocyst development rate in the multiPN group was significantly higher than in the MPN group ($p<0.001$). When the association between abnormal PN incidence and patients' characteristics was analyzed, no significant differences were found in the MPN/multiPN incidence when donor women were divided into age groups <35 years and ≥35 years of age ($p>0.05$) (Table 5).

The rates of abnormal PN between groups stratified by etiology of infertility was also investigated. No significant difference was found between the rates of multiPN among the infertility etiology groups by post-hoc analysis ($p>0.05$). The rate of MPN in the poor ovarian reserve group was significantly higher than those of the other etiological groups ($p<0.05$).

Table 2. Fertilization and blastocyst development among the study group

		n	**%**
Fertilization	No	540	29.7
	Yes	1280	70.3
PN (n=1280)	MPN	146	11.4
	2PN	1064	83.1
	MultiPN	70	5.5
Blastocyst development (n=1280)	No	578	45.2
	Yes	702	54.8
PN: Pronucleus number, MPN: Multiple pronuclear			

Discussion

The current study demonstrated that most of the embryos developing after MPN and multiPN did not reach the blastocyst stage. The proportion of those that reached the blastocyst stage was higher in multiPN embryos than in MPN embryos. The development of blastocyst from embryos with two pronuclei was significantly higher when compared to the other two groups with abnormal fertilization. According to this data, selection of embryos with an abnormal number of pronuclei for transfer is not recommended in routine practice. However, a small number of embryos that fertilized with an abnormal number of pronuclei in the oocyte can reach the blastocyst stage in some patients. Transfer of these embryos should be considered carefully.

This was a retrospective study and we evaluated patients having both normal and abnormally fertilized embryos. As patients already had 2PN embryos, further tests including trophoectoderm biopsy and PGS were not performed for abnormal PN embryos which reached the blastocyst stage to check the possibility of self-correction. In addition, blastoscysts developing from abnormal PN embryos were not transferred and all transfers were performed with blastocysts developing from 2PN embryos. Therefore, final IVF outcome between abnormal and 2PN transferred blastocysts were not evaluated.

Chromosomal abnormalities are observed in 31.4% of the 3PN zygotes (17). Most of the embryos with multiPN are usually triploid and usually result in abortion (17). In our study, 42.8% of multiPN embryos reached the blastocyst stage. Despite this high rate, the risk of abnormality may not be estimated without genetic screening before embryo transfer.

Development potentials of MPN zygotes with a wide pronuclear area or diameter are similar to those for 2PN zygotes (18). This may be observed due to early fusion of the male and female pronuclei. Embryos with MPN and two PB can be transferred only if a PGS diagnosis of euploidy is obtained and there is no other available normal fertilized embryo (19). The risk of chromosomal abnormalities in cells with MPN after ICSI is clearly higher than those after IVF and there are arguments that these embryos should never be transferred (20,21).

A study of embryos with an abnormal pronucleus compared embryos with 0 PN and MPN to embryos with 2PN on the fifth and sixth day of development and found no significant difference (22). In another similar study, implantation was not observed following transfer of the embryos developing from 0 PN and MPN (6). The embryos developing after MPN had the lowest rates of reaching blastocyst stage in our study. There is no consensus on the transfer of embryos with MPN in the literature, due to the limited number of studies.

An abnormal pronucleus may be iatrogenic, due to the methods used in assisted reproductive techniques. The incidence of multiPN is increased in cases of short incubation of gametes and early clearance of cumulus cells in oocytes (23). Embryos from MPN after IVF have higher rates of development on day 3, 5 and 6 compared to embryos from MPN after ICSI (24). Aneuploidy increases with maternal age, and abnormalities that occur after meiosis, such as mosaicism and polyploidy, may occur at a similar rate in all age groups (25). Kang et al. (26) reported that MPN and 3PN incidence increased in hyperstimulated cycles. It is controversial whether factors other than oocyte and sperm may also have an effect on abnormal pronuclei formation.

Different techniques and methods have been investigated for the correction of abnormally pronucleated embryos. One of these procedures is removing the excess pronucleus, by enucleation, from the zygotes with 3PN. A diploid embryo, with two pronuclei, was obtained after the procedure (27). The main limitation of this practice was the decision as to which pronucleus to remove. Removal of the wrong pronucleus may cause the embryo to carry two sets of maternal or paternal chromosomes. In a related study, one pronucleus of

Table 3. Development of blastocyst according to pronucleus number

		Total	Development of blastocyte
		n	n (%)
PN	MPN	146	25 (17.1)
	2PN	1064	647 (60.8)
	MultiPN	70	30 (42.9)
PN: Pronucleus number			

Table 4. Generalized Linear Mixed Models for the effect of PN number on blastocyst development

	Model		
	Beta	Exp (beta) (95% CI)	p
PN (MPN)	-	1	-
PN (2PN)	2,148	8,570 (5,405; 13,588)	**<0.001****
PN (multiPN)	1,504	4,501 (2,294; 8,828)	**<0.001****
MPN group is used as reference for PN variable. PN: Pronucleus number, CI: Confidence interval			

Table 5. Generalized Linear Mixed Models for the effect of age on PN number

	MPN	2PN	MultiPN	F, p
	n (%)	n (%)	n (%)	
Age				**F=2.266**
<35	94 (10.5)	744 (83.3)	55 (6.2)	p=0.104
≥35	52 (13.4)	320 (82.7)	15 (3.9)	
PN: Pronucleus number				

3PN zygotes was removed in the first group, and they were compared with a second group with 3PN zygotes followed without performing any procedure (28). The enucleation group had an increased potential for embryo formation, although the rate was still low when compared with 2PN zygotes (28). When karyotype analysis was performed on part of the enucleated group, 44.4% were identified as diploid and 55.5% as aneuploid.

Abnormal embryos can also be used to obtain human embryonic stem cells (hESC). hESC are cells with potential for self-renewal and differentiation into three germ layers (29). They can be used as a renewable source in cell transplantation for serious degenerative diseases. Huan et al. (30) obtained hESC from embryos with abnormal pronuclei, including 0 PN, MPN, and 3PN, and a normal pronucleus complement (2PN). All hESCs had normal chromosome content according to karyotype analysis. There was no structural difference among the obtained hESC cells, demonstrating distinct identity and karyotypic stability (30). Therefore, abnormal fertilized embryos can be used as a source for healthy hESC production.

However, genetic disorders can be observed in embryos developing after abnormal pronucleus formation. An embryo with mosaicism can correct itself by moving the mosaic cells towards the trophoblast during the development process, through the self-repairing mechanism of the cells (31). Tetraploid cells may be excluded from the primitive ectoderm lineage at an early stage. Another self-correcting mechanism is apoptosis and chromosomally abnormal embryos may undergo apoptosis (32).

A time-lapse system provides valuable morphokinetic data from embryo development in assisted reproduction techniques and each IVF laboratory should determine its own embryo selection criteria based on its own data (33). There is no consensus in the literature, whether abnormally fertilized embryos detected by time-lapse system are suitable for transfer.

Staessen et al. (5) reported the birth of two healthy children and one biochemical pregnancy after transfer of abnormally fertilized embryos. Grass and Trounson similarly recorded a healthy newborn in their study (34). One of the major successes was the birth of nine healthy infants from the transfer of embryos from MPN after IVF and implantation was observed in four of the embryos developing from MPN after ICSI (24). It is not right to declare that abnormally fertilized embryos should not be transferred. However, all abnormally fertilized embryos that have reached the blastocyst stage may not be eligible for transfer. In cases when there is no other embryo reaching the blastocyst stage, they can be transferred if no abnormality is detected after preimplantation genetic testing for aneuploidy (PGT-A).

Mutia et al. (35) analyzed 30 embryos developing from 3PN using next generation sequencing. They detected a normal chromosomal array in one third of them while the rest had abnormal chromosomes with the highest percentage of abnormality being triploidy (35). In vitro culture and chromosomal analysis of clinically discarded human embryos was investigated by Yao et al. (36). Blastocyst formation rates of 2PN embryos were higher than those for the abnormal 1PN, 3PN, and ≥4PN embryos (36). Lim and Lee (37) reported three healthy live births, following PGT-A and transfer of four euploid, 0PN-derived blastocysts to four patients. In addition, one on-going pregnancy was achieved by transfer of four euploid 1PN-derived blastocysts to four patients (37). In a similar study, Capalbo et al. (38) reported three live births by transfer of abnormally fertilized, oocyte-derived blastocysts after performing PGT-A. Hondo et al. (39) evaluated the rates of clinical pregnancy and live birth following transfer of frozen-thawed 1PN- and 0PN-derived blastocysts. The pregnancy and live birth rates for 0PN-derived embryos obtained by ICSI were similar to 2PN-derived blastocysts. However, 1PN-derived blastocysts had significantly lower rates (39).

Study Limitation

This study included some limitations. The status of the oocytes was only assessed on the first and fifth days. All data were analyzed from one center using a single protocol which was applied until day 5. Therefore, there is no data from day 6 onwards. In addition, if more extensive information from each day until the fifth day was available, there would have been a more detailed evaluation of the development of embryos after abnormal fertilization including factors such as pronucleus numbers, stages, and abnormalites in cell numbers until they reach the blastocyst stage. Scoring the embryos of MPN and multiPN origin reaching the blastocyst stage and comparing them with embryos of 2PN origin may further illuminate data on the quality of abnormally fertilized embryos.

Conclusion

Rate of blastocyst development after 2PN is significantly higher compared to those of the abnormal pronucleated embryos. MultiPN embryos have a higher chance to develop into blastocytes than MPN embryos. Large-scale research is difficult in this subject, because abnormal fertilization is not very common. Development of abnormally fertilized embryos usually stalls without reaching the fifth day. Owing to the low prevalence of abnormal fertilization, multicenter studies would delineate the effect of abnormal fertilization on embryonic development and provide more robust information about selection of abnormally pronucleated embryos for possible transfer.

Ethics Committee Approval: *The study protocol was approved by the Institutional Review Board of the Maltepe University Faculty of Medicine (approval number: 78, date: 22.09.2017).*

Informed Consent: *Consent forms were obtained from the patients.*

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Comparison of glucose degradation product and receptor levels in diabetic and normal pregnancy

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Abstract

Objective: The aim of this study was to assess the diagnostic values of new biochemical markers that may be an alternative to the oral glucose tolerance test (OGTT) and determine the differences in these markers among three groups of women with varying degrees of glucose homeostasis dysregulation.

Material and Methods: This was a prospective study. All women were screened with 50 gram (g) oral glucose and a 100 g OGTT for gestational diabetes mellitus (GDM). The patients were divided into three groups depending on the result of the tests: no evidence of glucose metabolism abnormality (controls); impaired glucose tolerance (IGT); and GDM. All three groups were evaluated for serum human advanced glycation end-products (AGEs) concentrations, carboxymethyl lysine (CML) concentration and receptor for advanced glycation end-product concentrations (RAGE/AGER), body mass index (BMI), age, fasting glucose levels, obstetrical parameters and gestational age.

Results: The study included 180 women divided into 59 (32.8%) GDM, 50 (27.8%) IGT and 71 (39.4%) controls. Age was similar among the three groups. Whereas fasting glucose levels and BMI in the three groups was significantly different, AGEs, CML, RAGE/AGER levels were found as significantly different between the groups ($p<0.001$).

Conclusion: In this study the use of AGEs, CML, and RAGE/AGER concentrations for the diagnosis and screening of gestational diabetes was investigated. It was found that advanced glycation products were significantly elevated in pregnancies with both IGT and GDM. These biochemical markers of glucose homeostasis dysregulation may have potential for GDM screening in the future. (J Turk Ger Gynecol Assoc 2021; 22: 127-31)

Keywords: Gestational diabetes mellitus, RAGE/AGER, CML, impaired glucose intolerance

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Introduction

There is no complete consensus on the diagnosis, screening, follow-up and treatment of gestational diabetes mellitus (GDM). GDM is the leading cause of maternal and fetal morbidity and perinatal mortality; the rates of fetal loss and illness in GDM pregnancies are about four times higher compared to normal pregnancies (1). The world's most common screening test is 50 gram (g) (1-hour) glucose test and its sensitivity is between 60% and 80%. A 100 g oral glucose tolerance test (OGTT) is used as a diagnostic test. Impaired glucose tolerance (IGT) has been shown to be an inter-metabolic disorder, between normal glucose tolerance and diabetes (prediabetes). IGT is a major risk factor for DM and it has been shown that, within 10 years, progression to full diabetes is between 20-50% ratio (2). Regulation of blood sugar levels following a GDM diagnosis during pregnancy prevents fetal macrosomia, which is associated with shoulder dystocia, birth trauma, and increased

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rate of cesarean section. In addition, metabolic complications such as hypoglycemia, polycythemia, hypocalcemia and hyperbilirubinemia, which occur in the neonatal period, can be prevented to a not insubstantial extent and maternal and fetal morbidity and mortality can be reduced (3).

Although there is some controversy concerning the definition of GDM, there is a close relationship between GDM and perinatal morbidity and mortality. Therefore, accurate and timely diagnosis and treatment have gained importance. OGTT has been used for the diagnosis of diabetes for years. OGTT criteria are frequently updated by organizations such as the American Diabetes Association (ADA) and the World Health Organization (4,5).

The development of quick, easy, cheap and reliable methods has become essential in diagnosis. There has been considerable research into new parameters that will contribute to the screening of diabetes. This current research includes studies on the utility of ketonic bodies, glycosylated Hb, fructosamine, microalbumin and advanced glycation products to this end. Protein glycation and advanced glycation products play an important role in the development of diabetic complications, which include retinopathy, nephropathy, neuropathy, rheumatoid arthritis, cardiomyopathy, and osteoporosis. The accumulation of glycation end products on proteins and free amino acids has been associated with diabetic vascular, renal, retinal, and neural complications (6). At first, this non-enzymatic process is reversible, but later it becomes irreversible. The increase in circulating glycation end products may lead to renal insufficiency, and excretion of the metabolites in the urine. If the glycation end products accumulate in tissues, renal and microvascular complications can occur due to crosslinking with collagen. There is a need for more evidence before clinicians can routinely use these new diagnostic parameters as an alternative to OGTT. To this end, we investigated whether or not advanced glycation products have a role in IGT or GDM.

Material and Methods

Pregnant women, between 24-28 weeks of gestation who were being followed in the department of obstetrics and gynecology of a tertiary hospital, were taken to the clinical biochemistry laboratory for GDM screening, as recommended in the 2003 ADA guidelines (7). Fifty grams of glucose was given orally, at any time of day without consideration of fasting. One hour after glucose was administered, plasma glucose was measured by the glucose oxidase method. In this study, 140 mg/dL (7.8 mmol/L) was used as a threshold value for blood sugar in the plasma, as suggested by ADA and ACOG (5,7). Following the administration of 50 g of oral glucose, if blood glucose level at the first hour was above this threshold, an OGTT using 100 g glucose was requested for definitive diagnosis.

Pregnant women who had type 1 and type 2 diabetes, multiple pregnancies, diagnosed endocrinopathies, kidney and liver disease, were under 18 years old, and pregnant women and who used drugs that could affect insulin secretion or susceptibility were excluded from the study group.

After 100 g OGTT, 95 mg/dL (5.3 mmol/L) of fasting glucose, 180 mg/dL (10 mmol/L) at one hour, 155 mg/dL (8.6 mmol/L) at the second hour and 140 mg/dL (7.8 mmol/L) at the third hour were used as the diagnostic criteria, as described by Carpenter and Couston (8) and Coustan (9), and GDM was diagnosed if any two values exceeding the threshold value. If any single value exceeded one of these threshold values, IGT was diagnosed.

The pregnant women who accepted to participate in the study were divided into three groups based on the diagnosis following the GDM screening tests. These groups were the GDM group, IGT group and a control group in whom the glucose concentration had remained below cut-offs at all time points. Then, fasting serum human advanced glycation end-products (AGEs), carboxymethyl lysine (CML) and advanced glycation product receptor (RAGE/AGER) levels were determined by ELISA methods, based on the antibody sandwich model.

All women gave informed consent to take part in the study. The study protocol was in compliance with the Declaration of Helsinki and approved by the local ethic committee. This study was supported by the Scientific Research Projects' Unit with the number of 2015TF/T-211.

Statistical analysis

SPSS, version 15.0 (IBM Inc., Armonk, NY, USA) program was used for statistical analysis. The distribution of data was evaluated by Kolmogorov-Smirnov method. ANOVA was used for comparison of normally distributed parameters and Kruskal-Wallis test was used for the comparison of non-normally distributed parameters. Data are presented as mean ± standard deviation. Chi-square test was used for comparison of qualitative data. The results were evaluated as 95% confidence interval and significance was assumed when $p<0.05$.

Results

In total 180 pregnant women were included in the study. These were divided into three groups consisting of: 59 (32.8%) GDM; 50 (27.8%) IGT; and 71 (39.4%) with no blood glucose metabolism impairments who constituted the control group. The groups did not differ statistically in terms of age, gestational week, gravida, parity, and living and abortus number. There was no statistically significant difference in diastolic blood pressure ($p=0.178$) but systolic blood pressure was statistically significant between the groups ($p=0.002$) (Table 1).

Fasting blood glucose (FBG) concentration, BMI values, occurrence of GDM in previous pregnancy and family history

of DM were statistically significantly higher in the GDM group (p=0.001). RAGE/AGER concentration differed significantly between the groups (p<0.001). Pairwise group comparisons of the RAGE/AGER concentrations showed significant differences. CML and AGE levels between the control group, IGT and GDM groups were significantly different (p<0.001) (Table 2).

Discussion

In this study, the 50 g OGTT and 100 g OGTT tests performed between January 2015 and January 2016 in our hospital were compared and examined the levels of glucose degradation products and receptors in pregnant women diagnosed with GDM and IGT in IGT by comparison to a control group with no evidence of impaired glucose homeostasis in pregnancy. Our aim was to investigate whether glucose degradation products, including AGE, RAGE and CML, are useful for the early diagnosis of IGT or GDM. There was a significant difference in the levels of the markers between the three groups. In the literature, there are no other studies investigating the relationship between glucose degradation products and IGT and GDM.

The risk of diabetes in women at age 30 is 4.4% whereas at age 35 it is 6.5%. In our study, the mean age of diabetic group was 32.34±5.43 years, which was insignificantly older than the control group (30.93±3.43; p>0.05). In Dornhorst and Rossi's (10) study, age was accepted as one of the unchangeable risk factors. In our study, when we compared age groups, we found that patients with negative screening test were statistically younger than those with GDM and IGT group (p<0.0001). There was no statistical difference between the other groups. This finding confirms that older age is a risk factor for GDM (11).

As the number of pregnancies increased, there was no significant difference in diagnosis of GDM by 100 g OGTT. It has been shown that the number of pregnancies is not among the risk factors for GDM (International Diabetes Study group) (12). Of those questioned about diabetes in family history, 51.1% had a positive diabetes story. In the absence of DM family history, the risk of diabetes is 4.4%, while in cases of positive DM family history the risk increases to 8.8% (13). There is a risk of recurrence of GDM in subsequent pregnancies. In a prospective study, GDM repeated in 47 of 90 (52%) pregnancies with GDM (14,15). In our study, 16.2% of the cases had GDM history in previous pregnancies.

The risk is 3.6% when BMI is 22 while it increases to 8.6% when BMI is 25. The risk also increases in positive family history with

Table 1. Clinical and anthropometric characteristics of the study groups

Parameters	Control (n=71)	IGT (n=50)	GDM (n=59)	p
Age (years)	30.93±3.43	32.92±5.46	32.34±5.43	0.059
BMI (kg/m^2)	27.58±4.64	30.98±4.63	33.43±5.96	<0.001
Gravida	2 (1-6)	3 (1-7)	2 (1-5)	NS
Family history of DM	2.8%	20.9%	28.8%	<0.001
History of GDM (%)	2.8%	20.9%	28.8%	<0.001
Gestational age (week)	26.5±1.3	27.2±1.8	27.4±1.7	0.689
Systole (mmHg)	112.01±13.71	116.96±16.71	121.36±15.02	0.002
Diastole (mmHg)	70.75±14.30	73.40±10.61	74.58±9.88	0.178

Data presented as mean ± standard deviation, median [IQR] or proportions. Differences evaluated by non-parametric chi-square test, respectively.
IGT: Impaired glucose tolerance, GDM: Gestational diabetes mellitus, BMI: Body mass index, DM: Diabetes mellitus, NS: Non-significant

Table 2. Laboratory characteristics of the study groups

Parameters	Control (n=71)	IGT (n=50)	GDM (n=59)	p
Fasting blood glucose (mg/dL)	86.79±14.43	99.76±30.28	104.58±38.36	0.001
RAGE/AGER (pg/mL)	676.19±97.51	840.35±182.85	954.29±216.24	<0.001
CML (pg/mL)	433.01±57.49	530.14±100.74	865.60±174.70	<0.001
AGE (pg/mL)	69.91±8.84	82.78±12.46	93.99±16.70	<0.001
50 g OGTT blood glucose (mg/dL)	118.17±25.82	157.10±11.63	173.20±20.60	<0.001
100 g OGTT blood glucose 0.h (mg/dL)	-	94.74±8.46	104.50±12.48	<0.001
100 g OGTT blood glucose 1.h (mg/dL)	-	171.20±11.20	189.67±21.62	<0.001
100 g OGTT blood glucose 2.h (mg/dL)	-	147.54±11.82	168.91±21.93	<0.001
100 g OGTT blood glucose 3.h (mg/dL)	-	118.06±17.85	140.53±21.39	<0.001

Data presented as mean ± standard deviation.
IGT: Impaired glucose tolerance, GDM: Gestational diabetes mellitus, RAGE/AGER: Receptor for advanced glycation end-product concentrations, CML: Carboxymethyl lysine, AGE: advanced glycation end-products, OGTT: Oral glucose tolerance test, g: Gram

the weight gain of the person. In our study, 100 g OGTT results were worse in women with high BMI. Dudhbhai et al. (16) also found that elevated BMI was more common in pregnancies with poor 100 g OGTT results. In our study, we found that the fasting blood sugar of the first application was higher in 100 g OGTT defects and this value was statistically significant ($p<0.05$). Perruchini et al. (17) also reported that the initial FBG was high in bad OGTT retrospectively.

The chronic hyperglycemia of diabetes leads to the formation of heterogeneous compounds known as AGEs. AGEs are formed as a result of non-enzymatic glycation of macromolecules, such as amino acids, proteins, peptides, phospholipids and nucleic acids. Diabetes-associated AGEs accumulate in tissues and lead to micro- and macro-complications of diabetes. Currently the structure of many AGE species has been clarified and it has become possible to identify them by using various properties (18-23). Hemoglobin A1c (HbA1c) is one of the endogenously formed glycation products, resulting from the glycation of the Hb molecule. HbA1c concentration is not only used to diagnose diabetes but it is also used for follow-up in diabetic individuals and indirectly informs about blood glucose levels over the preceding 8-10 weeks. Without doubt AGEs are more suitable for the evaluation of total glycation products as the test cannot distinguish between exogenously and endogenously glycated products of all proteins and nucleic acids. The AGE test also reveals glycation products formed by fructose and galactose (24,25).

The three groups were compared in terms of RAGE/AGER, CML and AGE values. The mean concentration of RAGE/AGER in the GDM group was significantly higher than that of the IGT group. Similarly, when CML levels were compared between the three groups this was found to be significantly higher in the GDM group compared with controls. A similar significant difference was found between the three groups for mean concentration of AGE with both the GDM and IGT groups having higher mean concentrations than the controls. The high CML and AGE levels in the GDM-diagnosed pregnancies supports similar results in previous studies (26-29).

AGE formation and its effects play an important role in the development of chronic complications of DM. A clear understanding of the structure of these heterogeneous compounds, standardization of measurement methods, and a clear understanding of the pathophysiology of long-term complications play a significant step in determining treatment options. Measurement of this emerging class of biomarkers may become a new tool in the monitoring the development of late complications from glycation in diabetic patients.

Conclusion

Currently, gestational diabetes screening, diagnosis, and follow-up methods are controversial. The significant differences in the mean levels of RAGE/AGER, CML and AGE in this study may indicate a use for them in future assessment of GDM. This will require further standardization of measurement protocols which may emerge following larger, prospective studies of utility in diabetes in general and in women being screened for GDM. Our results suggest that AGE, RAGE and CML values may contribute to early GDM diagnosis, but there is a need for extensive studies to determine sensitivity, specificity and suitability based on cost analysis in order to use these parameters in screening and diagnosis.

Ethics Committee Approval: *The study protocol was in compliance with the Declaration of Helsinki and approved by the local ethic committee.*

Informed Consent: *All women gave informed consent to take part in the study.*

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Ovarian stimulation drugs alter the metabolite content of the growing follicle: in vivo spectroscopic evaluation of follicle fluid

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Abstract

Objective: To determine and compare metabolite content using magnetic resonance spectroscopy (MRS) of growing follicles in patients with polycystic ovary syndrome (PCOS) receiving recombinant follicle stimulating hormone (rFSH), clomiphene citrate (CC) or aromatase inhibitor (AI) for ovarian stimulation.

Material and Methods: Thirty patients diagnosed with PCOS and infertility and scheduled for ovarian stimulation were divided by therapy, rFSH/CC/AI, into three equal groups. Five fertile cases were designated as the control group. When the follicle diameters reached 16-18 mm in each group, patients underwent MRS and the metabolite content of a dominant follicle was analyzed. N-acetylaspartate, lactate (Lac), creatine (Cr), and choline (Cho) metabolite levels in parts per million were measured in the spectra.

Results: A ~three-fold decrease in dominant follicle Cho content was found in patients receiving CC compared to control subjects. Similarly, the dominant follicle Cho intensities of patients given rFSH and AI were noted to be significantly higher than those who received CC. Only dominant follicle Lac levels of the patients who received CC were found to be significantly higher than the other groups. Cr peak intensities of patients receiving CC were found to be approximately three times less than control subjects. Cr signal intensity was significantly higher in patients receiving rFSH or AI than in patients receiving CC. While two patients became pregnant in the CC group, three patients in the AI group and five patients in the rFSH group became pregnant. The main metabolites detected in patients who conceived in each group were Cho and Cr. In cases who could not conceive, while Lac and lipid signals increased, Cho and Cr signals decreased.

Conclusion: Unlike CC, ovarian stimulation with rFSH or AI does not alter dominant follicle metabolite content. The developmental capacity of a growing egg may be determined non-invasively with MRS. (J Turk Ger Gynecol Assoc 2021; 22: 132-8)

Keywords: MR spectroscopy, rFSH, aromatase inhibitor, clomiphene citrate, dominant follicle

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Introduction

Drugs such as recombinant follicle stimulating hormone (rFSH), clomiphene citrate (CC) and aromatase inhibitor (AI), which are used for ovarian stimulation, have all the features necessary for the selection, growth and numerical increase of eggs. However, sometimes these drugs may lead to the formation and selection of poor quality oocytes, resulting in poor quality embryos (1-4). Letrozole, an AI, has a reversible effect and can block estrogen synthesis from androgens (4). While rFSH directly promotes follicle development, CC and AI stimulate follicle growth indirectly by increasing endogenous FSH release. Both letrozole and CC provide adequate follicle development when used for five days between 3rd and 7th days of the cycle.

However, in addition to the number of retrieved oocytes, both the quality of oocytes and the pregnancy rates obtained

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with these drugs are different (1). The main reason for this difference between induction drugs may be changes in follicle metabolism and development depending on the drug dose and mechanism of action. Both the agonistic and antagonistic effects of CC on estrogen receptors may affect follicle developmental capacity independently of endogenous FSH levels. Likewise, the aromatase enzyme inhibitory effect of letrozole may disturb estrogen production capacity of granulosa cells that may, in turn, lead to unfavorable consequences on follicle developmental potential (1-4). In addition, use of these drugs may also affect the morphological and receptive properties of the endometrium (1,2). However, there are not enough clinical data regarding the impact of these drugs on developing follicle metabolite composition. Clinicians therefore have to act on the basis of clinical and embryological data and experience rather than molecular data when deciding on which group of drug to use.

It is not possible to make a clear comment about follicle quality based on images obtained during folliculometry. For this reason, there is a need for a method to evaluate the developmental potential of the follicle non-invasively during ovarian stimulation cycles. A study by Wallace et al. (5) reported a positive correlation between follicle fluid metabolite content and oocyte developmental capacity. However, this study used invasive techniques because the follicle liquid was removed for in vitro analysis. Therefore, an oocyte pick-up (OPU) was required, which is an invasive procedure. In addition, since a nuclear magnetic resonance process was performed after OPU, there was no possibility of leaving the developing egg in vivo and undisturbed. To eliminate all these disadvantages and to evaluate egg development in vivo, the only non-invasive method available is magnetic resonance spectroscopy (MRS).

It has been reported that MRS can detect metabolite content in biological fluids with great accuracy (6). MRS can analyze the metabolic changes in a living cell using conventional MR imaging. The primary advantage of this technique is its non-invasive nature and cost. MRS can measure various intrafollicular molecules that are present at low parts per million (ppm) levels. Although the method was originally developed for brain lesions, its use in reproductive organs, such as endometrium, myometrium, and ovary has been reported by many authors (5-8). This technique also allows comparison of follicular fluid metabolite levels in different treatment regimens in vivo and non-invasively. Although there are many studies investigating the metabolite concentrations in the reproductive organs (5-10), there are no published studies investigating the effects of ovarian stimulation drugs on follicular fluid metabolite compositon. Therefore, the primary outcome of the study was to investigate whether rFSH, AI, and CC have an effect on metabolite content of growing follicle in women with polycystic ovary syndrome (PCOS) undergoing ovarian stimulation. The secondary outcome was to compare the possible relationship between the metabolite intensities obtained from the dominant follicle and clinical pregnancy rate.

Material and Methods

The study was conducted in the BAU Göztepe Medicalpark Hospital, Clinic of In-vitro Fertilization. Thirty-five women (30 PCOS and five fertile controls) between the ages of 19 and 37 years with a body mass index (BMI) of 18-29 were enrolled the study. The PCOS group, with primary infertility and anovulation, was divided in three equal groups, depending on antagonist protocol with rFSH (n=10), or PCOS women receiving CC (n=10) or PCOS women receiving AI (n=10). The controls had similar ages and BMIs and did not receive any drugs for ovarian stimulation. The women in control group had at least one or more children. They had no history of clinical or laboratory findings of PCOS. Women in each PCOS treatment group met the diagnostic criteria for PCOS when at least two of the following three features was present (11): 1) Amenorrhea or oligomenorrhea with chronic anovulation, 2) clinical and/or biochemical evidence of hyperandrogenism, and 3) ultrasonographic appearance of PCOS (2). Participants with a history of current or previous use of metformin, tubal or male factor infertility, evidence of any uterine, ovarian or peritoneal diseases, such as leiomyoma, benign ovarian cyst, endometrioma or endometriosis potentially affecting the ovarian microenvironment, were also excluded. This study has institutional review board approval.

PCOS participants on antagonist protocol were given daily rFSH starting from the third day of the menstrual cycle. Dose adjustments were performed according to BMI and ovarian response. The rFSH dose used ranged from 100 units to 187.5 units. Follicle development was monitored by using transvaginal ultrasonography and serum estradiol concentrations. As soon as one of the follicles reached 14 mm in diameter, gonadotropin-releasing hormone antagonist (cetrotide: 250 μg, Merck Serono, Turkey) was started and this treatment was continued until human chorionic gonadotropin (hCG) was administered. Oocyte maturation was triggered with recombinant hCG (ovitrelle, Merck-Serono, 250 mg, Modugno, Italy). As soon as the follicle diameters reached 16-18 mm in each group, patients underwent MRS and the metabolite content of a dominant follicle was analyzed. N-acetylaspartate (NAA), lactate (Lac), creatine (Cr), and choline (Cho) metabolite levels were measured in the spectra in ppm (10,12).

PCOS subjects in the CC group were treated with CC (serophene, Serono, Rome, Italy) at dosage of 150 mg/day for five days beginning from the third day of spontaneous or

progesterone-induced withdrawal bleeding. Patients in the CC group were selected from CC-resistant PCOS patients. CC resistance was defined as failure to ovulate after six months of treatment at an appropriate dose. In our study, patients who could not achieve ovulation despite using 150 mg/day clomiphene were considered CC resistant. PCOS subjects on AI group were treated with letrozole (Femara, Novartis, İstanbul, Turkey) at dosage of 2.5 mg/day for five days beginning from the third day of a spontaneous or progesterone-induced menstrual bleeding. Follicle development was monitored using transvaginal ultrasound (TV-USG). Women on CC or letrozole also underwent spetroscopic analysis of one dominant follicle as soon as the detection of a follicle with a mean diameter of at least 16-18 mm on TV-USG examination. Women in the CC or letrozole group underwent either timed intercourse or intrauterine insemination. Fertile women on their natural cycle underwent spectroscopy when the dominant follicle had a mean diameter of at least 16-18 mm.

Spectroscopic analysis of dominant follicle

Axial and coronal plane T1-weighted images of the dominant follicle with 5 mm thick sections were achieved using 1.5 T MRI. A single voxel MR spectroscopy procedure was applied with a short and long TE. As signals from other follicles may obscure the metabolite content of the dominant follicle, the selected voxel should be placed in an appropriate area so that it is possible to get clear information about the content of follicle fluid. Therefore, great care was taken to exclude neighboring follicles in the area of the selected voxel. Finally, the voxel was placed just in the middle of the dominant follicle, after ensuring no interference from neighbouring follicles or structures.

The metabolite ratios of acquired signals from the dominant follicle were determined using MR User Interface software Automated Quantitation of Short Echo Time MRS spectra (13). NAA, Lac, Cr, and Cho peaks of each group were analysed. A detailed discussion of these spectroscopic methods can be found elsewhere (6-9,12). The primary outcome of this study was to investigate the effects of rFSH, CC and AI treatment on dominant follicle metabolite levels, in terms of NAA, Lac, Cr and Cho. The secondary outcome was to compare the possible relationship between the metabolite intensities obtained from the dominant follicle and clinical pregnancy rate. All metabolite peaks were analyzed quantitatively and compared with results by either the timed intercourse, intrauterine insemination or embryo transfer methods. Clinical pregnancy rate was defined as evidence of a gestational sac confirmed by ultrasound examination.

Statistical analysis

Analyses of all data were performed on Statistical Package for Social Sciences (IBM Inc., Chicago, IL, USA). Q-Q and histogram plots or Kolmogorov-Smirnov test were used to determine whether variables were normally distributed. Data are given as mean ± standard deviation or median for continuous variables according to normality of distribution. Normally distributed variables were analyzed with the Independent samples t-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Categorical variables were analyzed with chi-square tests. A $p<0.05$ was considered to be significant.

Results

The clinical and demographic characteristics of the four groups were similar (Table 1). Three participants were excluded from

Table 1. Demographic characteristics and metabolites ratios of treatment and control groups

	rFSH	Clomiphene	Letrozole	Fertile control
Mean age (yrs)	29.1±6.31	28.3±1.40	28.6± 0.21	29.2±2.45
BMI (kg/m^2)	27.4±5.14	26.5±5.32	27.1±2.11	27.5±3.30
Infertility duration (yrs)	7.11±1.33	6.74±2.10	6.91±2.03	NA
Etiology of infertility	PCOS	PCOS	PCOS	NA
Mean follicle size (mm)	18.4±0.3	17.4±0.3	18.2±2.6	16.8±1.2
Method	IVF/ICSI	IUI or TIC	IUI or TIC	NA
Dominant follicle metabolite composition				
1. NAA	1.18 (0.9)	1.32 (1.1)	1.40 (0.8)	1.10 (0.7)
2. Lactate	0.98 (0.80)	1.89 (1.2)*	0.98 (0.7)	0.89 (0.6)
3. Choline	1.94 (1.0)	0.61 (0.5)*	1.93 (1.2)	2.12 (1.3)
4. Creatine	1.91 (0.9)	0.84 (0.7)*	1.92 (1.2)	2.33 (1.3)

*: Compared to CC group there were statistically significant differences between the fertile control, rFSH, and letrozole groups in terms of Cho, Lac, and Cr metabolites.
rFSH: Recombinant follicle stimulating hormone, BMI: Body mass index, IVF: In-vitro Fertilization, ICSI: Intracytoplasmic sperm injection, ICI: Intrauterine insemination, TIC: Timed intercourse, NAA: N-acetylaspar, Cho: Choline, Lac: Lactate, Cr: Creatine, PCOS: Polycystic ovary syndrome, NA: Not available

the study due to inadequate follicle growth in one fertile control and bad spectral image in two women receiving letrozole. The remaining 32 subjects in control and treatment groups had good spectral images. Compared to the fertile group, a significantly decreased Cho signal was found in the CC group. An approximately three-fold decline in Cho peak intensity was found in PCOS subjects compared to the Cho peak intensity of control participants [0.61 (0.5) ppm vs. 2.12 (1.3) ppm, respectively; $p<0.01$]. Likewise, the Cho peak intensity in the rFSH and AI groups were higher than those in the CC group. The Cho peak intensity of fertile controls and patients receiving rFSH or letrozole were similar.

The Lac signal intensity of patients receiving CC was significantly higher than that of fertile controls [1.89 (1.2) vs. 0.89 (0.6), $p<0.02$] or patients receiving rFSH [0.98 (0.80) vs. 1.89 (1.2), $p<0.03$] or letrozole [0.98 (0.7) vs. 1.89 (1.2) $p<0.01$]. An almost two-fold increase in Lac signal was detected after CC treatment compared to rFSH, letrozole or fertile groups. In contrast, Lac signal intensities of fertile controls, and the rFSH and letrozole groups were similar. Follicle fluid Cr signal intensity of fertile group was approximately three times higher than Cr signal of patients receiving CC [2.33 (1.3) vs. 0.84 (0.7) $p<0.01$]. Likewise, Cr signal intensity of women receiving rFSH or letrozole was significantly higher than those receiving CC ($p<0.01$ and $p<0.01$, respectively). No significant difference was found between the fertile control, rFSH, and letrozole groups in terms of Cr metabolite. The NAA content of fertile and treatment groups were found to be similar (Table 1 and Figure 1).

When a comparison was made between follicular fluid metabolite signal intensities and pregnancy rates, the following results were obtained. While clinical pregnancy was detected in 2 of 10 patients in the CC group, pregnancy could not be achieved in the remaining eight cases. While the most prominent metabolites were Cho and Cr in the two cases who became pregnant, in those who could not conceive, the Lac peak was most prominent. While pregnancy was achieved in 3 of 10 cases in the AI group, pregnancy was not detected in seven cases. While high Cho signal was detected in pregnant patients, weak Cho signal and high lipid signal were detected in those who could not conceive. While 5 out of 10 patients in the rFSH group had clinical pregnancy, the remaining five cases did not become pregnant. High Cho and low Lac signal were typical findings in patients who became pregnant in this group. Weak Cho and Cr signal was striking in patients who could not conceive.

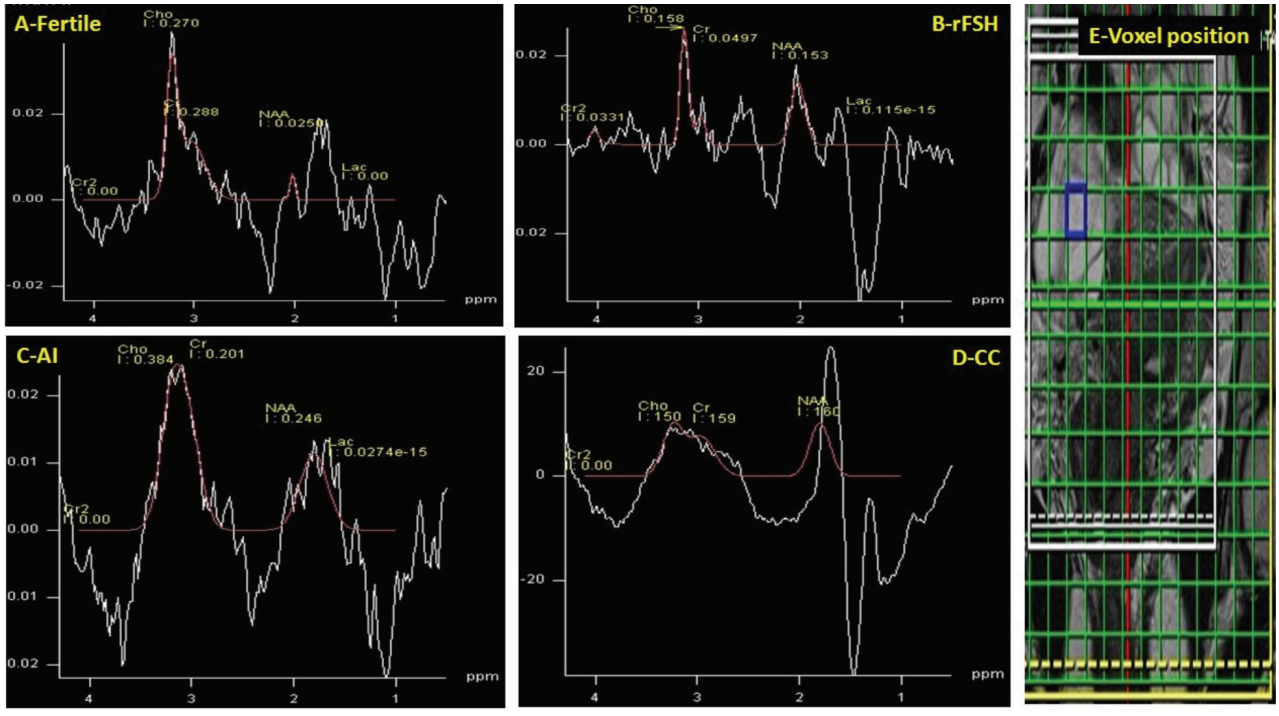

Figure 1. Spectroscopy analysis of fertile group (A) and participants receiving rFSH (B), aromatase inhibitor (C) or clomiphene citrate (D). An approximately four-fold decrease in Cho signal was detected in PCOS group receiving CC (D). Cho signal of subjects taking rFSH or AI were significantly higher than in the CC group. Signals from ovary and other pelvic structures may obscure the metabolite resonance of dominant follicle. Hence, the voxel was placed at the center of dominant follicle to avoid any signal contamination from neighboring tissues (E)

rFSH: Recombinant follicle stimulating hormone, NAA: N-acetylaspartate, Lac: Lactate, Cr: Creatine, Cho: Choline, AI: Aromatase inhibitor, CC: Clomiphene citrate, PCOS: Polycystic ovary syndrome

Discussion

If a technique makes it possible to determine the best quality oocyte before OPU, pregnancy rates could be improved. Although morphological analysis of the oocyte is the basic method for determining embryo quality, it does not always correctly detect reproductive outcome (14,15). Metabolite composition of developing follicle is an indicator of whether an embryo will complete its developmental process and reach the cleavage stage or not (16,17). Although drugs used for ovarian stimulation improve maturation of oocytes, adverse effects of these drugs on natural selection steps can cause retrieval of developmentally incompetent oocytes. In good agreement with this, it has been reported that few of the embryos collected by OPU are competent to complete their developmental stages in a healthy manner and gain implantation potential (17). Fortunately, there is a new and non-invasive method to test the effects of ovarian stimulation drugs on the metabolite profile of the developing follicle, MRS. MRS is an advanced technology that enables the determination of metabolite content in all biological fluid layers in detail. This non-invasive method has been used in the evaluation of reproductive tissues including ovary and endometrium (6).

Follicle fluid has a nutritive effect on the growing follicle (18) so determining the metabolites in this fluid non-invasively would provide preliminary information about the metabolite content of the developing follicle. Studies have demonstrated that measurement of Cho and Lac metabolites in follicle fluid with nuclear MRS correlates with oocyte developmental capacity (5,16). The metabolite composition of the cumulus oocyte complex in in vitro maturation cycles was described by Uhde et al. (19). After 23 hours of follow-up, 369 different metabolites were isolated in maturation medium. The metabolites which exhibited a significant increase in concentration during follicle growth include amino acids, saccharides, lipids, UDP-glucose, Lac, pyruvate and sphingomyelin. The two metabolites with the most significant increase in concentration were Cr and Lac. During 23 hours of maturation period, a 516-fold increase in Cr and a 1227-fold increase in Lac were reported. Lipids have a crtical role in energy production during oocyte maturation and the establishment of a developmentally mature oocyte (19). The transport of CC-derived pyruvate with lipids into the oocyte is critical for ATP production by the oocyte (20). Uhde et al. (19) showed that during oocyte maturation there was a significant increase in concentrations of both pyruvate and Lac in the medium, while glucose concentrations decreased. One possible reason for this decrease in glucose levels in follicle fluid would be increased glycolysis in cumulus cells during the maturation period.

In the current study, the effects of rFSH, CC, and AI on dominant follicle metabolite content were investigated for the first time. The main result was that the metabolite signals of growing follicle were quite different, depending on which treatment had been given, even though the follicle size was sufficient on ultrasonographic examination. The metabolite composition of dominant follicles of women receiving AI or rFSH were not different from those of fertile patients. On the other hand, the metabolite signals of patient receiving CC were different from that of fertile control, rFSH or AI groups. The Cho, Cr, and Lac signals obtained from subjects receiving CC were significantly different from the signals of rFSH and AI groups. Of the four measured metabolites, only NAA signals were similar between the groups. Additionally, the intensity of the Lac signal of the dominant follicle was elevated in the CC group compared with rFSH or AI group. Administarion of CC for ovarian stimulation disturbed the release of Cho and Cr that are basic metabolites produced by the developing follicle. The mechanism of impaired metabolite signals in patients using CC is not clear. This adverse effect may be due to the estrogen receptor blocking effect of CC. CC has been shown to have a negative impact on the endometrium (1). Despite high ovulation rates, low clinical pregnancy rates in women given CC may be due to failed follicle development. Both AI and CC stimulate follicle development by increasing endogenous FSH levels. However, AI reduces estrogen levels by blocking the aromatase enzyme and leads to an increase in FSH levels. CC increases FSH levels by binding to estrogen receptors. Since CC binds to all estrogen receptors, it also binds to estrogen receptors in cumulus cells. Thus, while CC contributes to follicle development via FSH, it also negatively affects follicle development by blocking estrogen effects in the cumulus cells. Since AI does not block estrogen receptors, it is unsurprising that different follicular growth patterns are seen in women treated with AI and with CC.

The increase in follicular fluid Lac levels may have both negative and positive effects on follicle development. High levels of follicle fluid Lac may lead to anaerobic glycolysis and impair healthy oocyte development in women on CC (6-8). Interestingly, exposure of developing mouse oocytes to a high fat diet results in failed oocyte nuclear maturation (21). Likewise, decreased levels of Cho metabolite in women on CC can be a sign of defective follicle development. Cr signal is an important indicator of energy metabolism of living cells (6-8). Low Cr signals in women receiving CC may be another indicator of impaired follicle maturation and can be associated with poor follicle morphology. In contrast, the increase in follicular fluid Lac levels in the CC group should not be interpreted as an absolute pathological state. Increase in follicular fluid Lac levels is also detected in natural follicle development

processes. Especially in in vitro maturation cycles, during the first eight hours of follow-up, a very significant increase in Lac concentration is detected in the maturation medium. It has been reported that the Lac concentration in the maturation medium increased 667-fold during in vitro maturation (19). What is important here is whether the oocyte is able to enzymatically transport Lac rather than the increase in fluid Lac levels, *per se*. Hérubel et al. (22) showed that oocytes highly express monocarboxylic acid transporters that enable the transport of Lac between cumulus cells and the oocyte. If the increase in Lac levels during follicle development exceeds the transport capacity of the available monocarboxylic acid transporters, the rate of cell growth and proliferation will slow down. Since the high Lac increase seen in the CC group is above the carrying capacity of the follicle, the cumulus cells will try to compensate for the Lac increase by slowing cell proliferation (7). Since Cho is a marker of cell membrane turnover, the amount of Cho passing into follicular fluid will also decrease due to decreased cumulus cell proliferation.

Conclusion

It is possible to predict the developmental capacity of an egg before OPU by determining metabolite content of a growing follicle specifically and accurately with spectroscopy. This is important as it would aid in selection of the best quality embryo and would aid in overcoming immature and incompetent oocyte selection. Thus, the selection of oocytes that have completed their developmental process before embryo transfer is possible and the transfer decision can be made accordingly.

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Premenstrual syndrome, a common but underrated entity: review of the clinical literature

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Abstract

Premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) are characterized by somatic and psychologic symptoms that arise at the luteal phase of the menstrual cycle and subside with menstruation. For definitive diagnosis prospectively self-reported symptoms should demonstrate a cyclic pattern and other psychological pathologies and thyroid dysfunction, that may present with similar symptoms, should be excluded. Both entities affect millions of women at reproductive age as the prevalence of PMS is given as 10-98% while PMDD affects 2-8%. Sex steroids and neurotransmitters have a central role in the etiology. The role of vitamins and minerals in the etiology and treatment of PMS and PMDD is open to discussion. Drugs that suppress ovarian sex steroid production, such as combined oral contraceptives or selective serotonin re-uptake inhibitors enhancing central serotonin delivery are used for treatment. Life-style changes and regular exercise also have a positive effect in milder cases. Tricyclic antidepressants and gonadotropin-releasing hormone analogues can be used in selected cases. (J Turk Ger Gynecol Assoc 2021; 22: 139-48)

Keywords: Premenstrual syndrome, premenstrual dysphoric disorder, etiology, treatment

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Introduction

Premenstrual syndrome (PMS) is an entity characterized by the presence of psychiatric symptoms such as mood swings, depression, loss of confidence, anxiety and irritability, without any underlying psychiatric disorder, accompanied by physical symptoms. Typical complaints include bloatedness and mastalgia encountered at the luteal phase of the menstrual cycle (LPMC), that deteriorates the well-being of the women and then subsides or disappears with menstruation (1). PMS affects a huge proportion of women at reproductive age and is characterized by the cyclic recurrence of a range of symptoms shown in Table 1 during the LPMC (2-4). Symptoms occur mostly in women of 25-35 years old, although it may be observed at any age between adolescence and menopause. Premenstrual dysphoric disorder (PMDD) is at the more severe end of the PMS spectrum that is characterized by the cyclic recurrence of psychological manifestations including irritability, nervousness, agitation, anger, insomnia, difficulty in concentrating, severe fatigue, depression, anxiety, and confusion. In addition, neurologic and vascular conditions are present which may include headache, dizziness, numbness, heightened sensitivity of arms and/or legs, palpitations, gastrointestinal and ocular symptoms that disrupt the daily life and functioning of the affected women. The mood disorder symptoms that are experienced both in PMS and PMDD disappear within the first days of menstruation. The Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) established seven criteria (A through G) for the diagnosis of PMDD and at least five of these symptoms should be present, and should include one of the first four (A-D) (5). Both PMS and PMDD show a cyclic pattern with affective, behavioral and somatic symptoms beginning at the LPMC and disappearing within a few days after the onset of menstruation. These cyclic symptoms can also be observed in amenorrheic, reproductive aged women who had a hysterectomy but have functioning ovaries.

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Epidemiology and prevalence

During the LPMC at least one physical or psychiatric symptom is observed in 80% of the women. However most of them do not report any change in their daily life and activities (6). In a study of 2800 French women 12% demonstrated the diagnostic symptoms of PMS while only 4% had more severe symptoms (7). Although most women experience one or a few of these symptoms during the LPMC, only 3-8% demonstrate clinically significant PMS symptoms (8).

Three prospective, population-based studies that utilized the strict criteria for diagnosis of PMDD reported a prevalence of approximately 2% among the study population (8,9).

In a study conducted at Switzerland in 2007, a total of 3,913 women aged 15 to 54 responded to a questionnaire inquiring about PMS symptoms and 3,522 (90%) reported that PMS affected their daily life. While 90% had at least one symptom, 10.3% had PMS, and 3.1% met the PMDD criteria (10).

Also, in a meta-analysis covering 18,803 women, the overall prevalence of PMS was 47.8% [95% confidence interval (CI): 32.6-62.9]. The lowest and highest prevalence were reported as 12% (95% CI: 11-13) in France and 98% (95% CI: 97-100) in Iran, respectively. The authors emphasized that the prevalence of PMS was studied more in Asia than in other continents (11).

A global study involving 7,226 women from South America, Europe and Asia, investigating the frequency of PMS symptoms and found the frequency to be parallel between countries and the regions, but in some countries, such as Pakistan, women were found to be less familiar with the term PMS compared to European women (12).

Risk factors for PMS and PMDD

The role of genetic factors in the predisposition to PMS and PMDD has been an active and interesting area for many researchers yet definitive conclusions have not emerged. Some studies suggest a possible association with *estrogen receptor alpha (ESR1)* gene (13-15). In one report, cells from women with and without PMDD appeared to show different response patterns to the components of the ESC/E (Z) complex containing the *ESR1* gene (16). Other risk factors for PMDD development include lower levels of education and smoking (17), history of traumatic events or anxiety disorder, and higher daily difficulty scores (18).

Etiology and pathogenesis

Although various hypotheses have been put forward, the etiology of PMS and PMDD is not fully understood (19). The best-known hypothesis is the presumed role of circulating gonadal steroids in the development of PMS symptoms, as suppression of ovulation has a beneficial effect on PMS (20-22). However, cyclic changes in ovarian steroids do not appear to be the only cause of PMS symptoms, as daily serum progesterone and estrogen concentrations are shown to be similar in women with and without PMS. An early study by Andersch et al. (23) demonstrated that, apart from prolactin levels, which were lower in the follicular phase of the PMS group, all the sex steroids were present in similar concentrations in both the control and the PMS groups in the LPMC. In two studies no correlation was found between serum progesterone levels and the affective and/or somatic symptoms (24,25). Deficiencies in progesterone, progesterone metabolites (some of which have anxiolytic effects) and progesterone receptor function have also been proposed to be a possible cause of PMS/PMDD. However, as mentioned, serum progesterone concentrations are normal in women who have PMS. In addition, serum concentrations of the progesterone metabolites allopregnanolone and pregnenolone are similar in women with PMS and without PMS (25). It has been shown that blocking the effect of progesterone in the LPMC with a progesterone receptor antagonist, such as mifepristone, does not alleviate PMS symptoms (26). Women with PMS may have an abnormal response to normal ovarian hormonal changes, even though serum progesterone and estrogen concentrations are within normal limits (21).

Current evidence suggests that PMS is a disorder triggered by changes in gonadal steroids during the LPMC in susceptible women. This is thought to be due to the interaction between cyclic changes in ovarian steroids and the functioning of central neurotransmitters. One of the most frequently investigated neurotransmitters in PMS pathogenesis is serotonin, but beta-endorphine, gamma-aminobutyric acid (GABA) and the autonomic nervous system are also part of the pathogenesis of PMS. Based on in vitro data and animal studies, there is evidence that cyclic variation in circulating estrogen and progesterone result in marked changes in the opioid (27), GABA (28) and serotonin (29) systems.

The potential role of the GABAergic system in PMS has not been extensively investigated previously. The main effect of GABA is to reduce cellular excitability through a chloride system. The hypothesis that suggests a modulatory role of progesterone in the GABAergic system is supported by the improvement observed in PMS symptoms when agents such as benzodiazepine and alprazolam, that increase GABAergic activity, are used (30). Additionally, GABA-A enhances receptor function and has anxiolytic effects. Low levels of the progesterone metabolite allopregnanolone are shown to produce a similar anxiolytic effect (31).

Current studies highlight the pivotal role of serotonin in the etiology of PMS. In a number of studies, patients with PMS have been shown to have lower levels of whole blood

serotonin, platelet serotonin, and imipramine binding and serotonin metabolites in the LPMC (32-36). In cerebrospinal fluid, 5-hydroxyindoleacetic acid has been shown to be present in higher levels compared to the dopamine metabolite homovanillic acid (37). In a study by Brzezinski et al. (38) and Menkes et al. (39), improvement in PMS symptoms was achieved with the serotonin agonist fenfluramine (38) and aggravated with acute reduction of the serotonin precursor tryptophan (39). In addition, selective serotonin reuptake inhibitors (SSRI) that increase the serotonin level in brain, such as fluoxetine, are one of the most effective drugs for treatment of PMS.

The role of minerals and vitamins in the etiology of PMS is still controversial. In previous studies, vitamin E, vitamin A or vitamin B6 levels were not different in women who have PMS (40,41). Several vitamins and dietary supplements have been studied as therapeutic agents for PMS, including vitamin E, B, vitex agnus castus, calcium, zinc, and magnesium. However, the evidence showing any of these are more effective than placebo is scarce. A Cochrane review that described a protocol for vitex agnus castus use for PMS was withdrawn (Cochrane Database of Systematic Reviews 2018, issue 3. art. no.: CD004632.). Also, although several studies related to iron, magnesium, zinc, zinc to potassium ratio, and calcium are present in the literature, their role in the treatment and etiology of PMS/PMDD is still debatable (41,42).

Diagnostic criteria for PMS and PMDD

Many groups have published diagnostic criteria for premenstrual diseases, including the World Health Organization, American College of Obstetricians and Gynecologists (ACOG), Royal College of Obstetricians and Gynecologists (RCOG), International Society for Premenstrual Disorders (ISPMD) and the American Psychiatric Association (APA; DSM-5) (1). ACOG describes symptoms consistent with PMS as: 1- The symptoms should be restricted to the LPMC; 2- The symptom pattern should be confirmed by prospective evaluation; 3- symptoms should cause functional impairment; and 4- other diagnoses that could better explain the symptoms should be excluded (43). The ACOG definition involves the presence of at least one of the six affective symptoms (angry outbursts, depression, anxiety, confusion, irritability and social withdrawal) and one of the four somatic symptoms (abdominal bloating, headache, breast tenderness, and swelling of extremities) reported five days prior to the onset of menses in the three prior menstrual cycles which have ceased within 4 days of onset of menses (43). ACOG has added more symptoms in the patient-available resources which include emotional symptoms such as depression, irritability, angry outbursts, crying spells, social withdrawal, anxiety, confusion, poor concentration, insomnia, increased nap taking, and changes in sexual desire, while physical symptoms include thirst and appetite changes (food cravings), weight gain, breast tenderness, bloating and headache, swelling of the hands or feet, aches and pains, abdominal pain, fatigue, skin problems, and gastrointestinal symptoms (https://www.acog.org/patient-resources/faqs August 2020).

The RCOG recommends keeping a self-reported symptom diary by the women for recording the symptoms for diagnosis of PMS and that the symptoms should be observed prospectively over two cycles (44).

ISPMD published a consensus article on the management of PMD and cited the classification published by O'Brien et al. (45) in 2011. According to this classification (46) two entities are described which are: 1) Core premenstrual disorder (CPD); and 2) Variant premenstrual disorders. The symptom characteristics of CPD can be somatic and/or psychological and were stated as occurring in ovulatory cycles, being absent after menstruation, and before ovulation, recurring in the luteal phase and be prospectively rated for at least two cycles. In addition, CPD symptoms should cause significant impairment of daily life (work, school, social activities, hobbies, interpersonal relationships, and distress). PMDD was defined as a sub-group of CPD. The variants of PMD were: a) Menstrual exacerbation of symptoms of an underlying somatic, psychological or medical disorder that significantly worsens premenstrually; b) PMD due to non-ovulatory ovarian activity; c) Progestogen-induced PMD related to exogenous progestogen administration; and d) PMD with absence of menstruation due to ongoing ovarian activity.

PMS is currently being diagnosed when any one of the four symptoms that may be physical, behavioral, or emotional/psychological (with at least one being emotional) or physical or behavioral and five or more symptoms are present. However, if a woman has five or more symptoms and one of them is an "emotional symptom", it would be better to diagnose PMDD instead of PMS (Table 1).

PMDD, classified by the APA, should include five of the 11 symptoms required to meet the diagnostic criteria, defined in DSM-5 and at least one of these should involve mood swings (5). Currently the APA DSM-5 system, that defines PMDD criteria, is used for diagnosis. These criteria specify that: a) prospective documentation of behavioral and physical symptoms (using diaries) for most of the previous year should be available; b) accompanied by five or more symptoms that occur in the week before the onset of menstruation and disappear within a few days of the onset of menstruation. These criteria also indicate that PMDD can overlap with other psychiatric disorders but in these patients the symptoms do not exacerbate in the luteal phase.

According to the DSM-5 criteria, one or more of the following must be present for the diagnosis of PMDD: a) anger/irritability b) sudden sadness, increased sensitivity, mood swings to rejection; c) tension and anxiety; and d) depressed mood, feeling hopeless, self-critical thoughts.

To achieve a total of five symptoms, one or more of the following symptoms must also be present:

a. Premenstrual irritability,
b. Concentration difficulty,
c. Appetite change, overeating, food cravings,
d. Decreased interest in ordinary activities,
e. Easily fatigued, having reduced energy,
f. Feeling overwhelmed or out of control,
g. Bloating, breast tenderness, weight gain, or joint/muscle pain,
h. Sleeping too much or not getting enough sleep.

These symptoms should occur in the luteal phase and must be severe enough to deteriorate daily functions (work, school, social life).

Table 1. Most frequent symptoms of premenstrual syndrome (2-4)

Most frequent dymptoms of PMS	
Behavioral symptoms	Fatigue
	Insomnia or needing more sleep
	Dizziness
	Sexual dysfunction
	Overeating
Psychologic symptoms	Nervousness
	Anger
	Depressive mood changes
	Anxiety
	Mood changes
	Difficulty in concentrating
	Confusion
	Forgetfulness
	Emotional sensitivity
	Poor self-esteem
	Emotional insensitivity
	Agitation
	Restlessness
Physical symptoms	Headache
	Mastodynia and mastalgia
	Backache and/or abdominal pain
	Bloating, weight gain
	Swollen ankles, hands and feet
	Nausea
	Muscle and joint pain

PMS: Premenstrual syndrome

Symptoms

Women with PMS experience a wide range of cyclic and recurrent emotional, physical, behavioral and cognitive symptoms that begin in the LPMC and resolve shortly after the onset of the menstrual period (follicular phase). The number of symptoms seen in the majority of patients is much more limited (47). However, core symptoms include emotional symptoms such as depression, irritability and anxiety, and somatic symptoms such as breast pain, bloating and swelling and headache (Table 1). For most women, the types of symptoms are consistent between periods and usually last an average of six days a month (48). Besides affective symptoms, women with PMDD, also have physical symptoms. Analysis of prospective symptom studies in women with PMDD shows that mood and physical symptoms are usually the most severe (and with functional impairment) within four days before menstruation in the first two to three days (49).

The most common emotional or behavioral symptom in PMS is mood swings. Other non-physical behavioral symptoms include irritability, anxiety/tension, sadness or depressed mood, increased appetite, sensitivity to rejection, and decreased interest in activities (47). The most common physical symptoms in PMS are abdominal bloating and excessive fatigue followed by breast tenderness, headache, dizziness and flushing (47). Hot flushes, similar to menopausal hot flushes, that occur before menstruation suggest PMS or PMDD in women who are not in postpartum or menopause (50).

Many symptoms associated with PMS significantly affect quality of life, ranging from a moderate to a severe effect and PMS symptoms have been associated with decreased health-related quality of life (51,52).

Differential diagnosis

For differential diagnosis of PMS and PMDD the entities given in Table 2 should be ruled out (Table 2) (43,44,46).

Physical examination

There is no specific finding on physical examination in women with PMS/PMDD. Physical examination might be useful in ruling out other entities, such as endometriosis.

Laboratory findings

There is no specific biochemical or any other tests to diagnose PMS. Daily gonadotropin and sex steroid serum concentrations are not different from women with and without PMS (23,24). Thyroid tests might be useful in order to exclude hypothyroidism.

General approach and treatment

A thorough evaluation of patients with PMS or PMDD is required (Table 3). The cyclic occurence of symptoms should be confirmed by careful questioning. It might be difficult to interpret in women who have PMS or PMDD with irregular menses. The etiology of the irregular menses should be investigated. In women who are using combined oral contraceptives the timing of the initiation of the symptoms and the relation to the onset or termination of the combined pill should be queried. Various screening tools or applications can be used for self-reporting the symptoms. In cases where the self-reported dairy is not conclusive, the RCOG recommends the use of GnRH testing. PMS can be diagnosed if the symptoms subside when ovarian hormonal suppression is obtained with GnRH analogues.

Treatment

The main goal of treatment for women with PMS/PMDD is to alleviate and improve symptoms and enable normal daily life. Various approaches, ranging from lifestyle measures (exercise and relaxation techniques), to cognitive behavioral therapy (CBT) and medications (SSRIs), and/or combined estrogen-progestin contraceptives (COC), are utilized for treatment of PMS and PMDD.

Table 2. Differential diagnosis of premenstrual syndrome and premenstrual disphoric disorder

Affective disorder (depression, anxiety, dysthymia, panic)
Anemia
Anorexia or bulimia
Chronic medical conditions (diabetes mellitus, hyperthyroidism, hypothyroidism)
Dysmenorrhea
Endometriosis
Hypothyroidism
Oral contraceptive pill or progestin-only contraceptive use
Perimenopause
Personality disorder
Substance abuse disorders

Table 3. Steps for evaluation of patients with PMS and PMDD

Take a detailed history of the symptoms and their relationship with the phase of the menstrual cycle
Take a medical history, including hormonal therapy and contraceptive drug use
Endocrine disorders should be evaluated and investigated (thyroid stimulating hormone)
If the symptoms are consistent with PMS/PMDD and other medical conditions are excluded, the patient should be asked to prospectively record symptoms for two months in order to confirm the diagnosis
PMS: Premenstrual syndrome, PMDD: Premenstrual dysphoric disorder

Treatment of mild symptoms

Regular exercise and stress reduction techniques are efficient and cost-effective treatment options in women with mild premenstrual symptoms. Exercise is particularly useful for physical symptoms (53).

Vitex agnus castus is a popular herbal remedy that is used as a dry extract, tincture or liquid for treatment of PMS symptoms. The mode of action and pharmacodynamics is not yet completely understood. In animal studies it is shown to act on the dopaminergic receptors leading to a reduction of prolactin secretion. Van Die et al. (54) reported vitex agnus castus to be more effective than placebo for PMS symptoms, while its effectiveness for PMDD is less prominent.

Although a range of vitamins and dietary supplements, including St. John's Wort, evening primrose oil, vitamin B6, vitamin E, vitamin D, zinc, calcium, iron and magnesium, have been studied as therapeutic agents for mild PMS, the evidence that demonstrates an increased effectiveness of these agents when compared to placebo is not sufficient (41,42,44,55).

Treatment of moderate and severe symptoms

A holistic approach conducted by a team consisting of gynecologist, clinical psychologist, psychiatrist or counsellor, and a dietician is required in treating women with moderate to severe PMS and PMDD (44). The best approach for women who meet the criteria of PMS or PMDD, is to have pharmacological and/or behavioral intervention, such as CBT. CBT aims to help people to identify and change destructive or disturbing negative thought patterns. However, before starting treatment, other conditions with symptoms that may overlap with PMS/PMDD, should be ruled out.

There are two targets for first-line pharmacotherapy of moderate to severe PMDD:

a. Enhancing central serotonergic delivery. Serotonin augmenting drugs alleviate PMDD symptoms, therefore SSRIs are highly effective in treatment of PMDD,

b. Suppressing the hypothalamic-pituitary-ovarian axis to eliminate physiologic cyclic changes of ovarian sex-steroids by either GnRH agonists or COCs.

SSRIs should be preferred in women who do not desire to use contraceptive pills. However, if the patient needs contraception, combined oral contraceptives should either be the first- choice or combined with SSRIs therapy.

SSRIs are reported to be effective in clinical trials and systematic reviews (44,56). A Cochrane review compared the

effectiveness of paroxetine, fluoxetine, escitalopram, sertraline and citalopram with placebo and concluded that SSRIs reduced overall self-rated symptoms significantly more effectively than placebo when either used continuously or during the LPMC (57). Paroxetine is also effective, but is associated with weight gain (58). The beneficial effect of SSRIs will occur as early as the first cycle. If the response is low, the dose may be increased before the next cycle. Although the Cochrane review demonstrated SSRI's therapeutic effect on both somatic and psychological symptoms, they appear to be more effective for mood symptoms than somatic symptoms (46).

The serotonin-norepinephrine reuptake inhibitor (SNRI), Venlafaxine, has also been reported to be more effective for PMDD than placebo, but SSRIs are still advised as first-line therapy because venlafaxine causes more withdrawal symptoms than SSRIs (59,60).

Tricyclic antidepressants can also be used. Clomipramine (given during the menstrual cycle or limited to the LPMC) is more effective than placebo, but its routine use is not recommended due to its side effects (sedation, dry mouth, and weight gain) (61,62).

Three protocols used for treatment of moderate to severe symptoms of PMS and PMDD by SSRIs are: continuous daily administration, luteal phase therapy, or symptom initiated therapy. The choice of regimen depends on the duration and timing of the symptoms (including their predictability) and the patient's preference.

1. Continuous use: SSRIs are effective for premenstrual symptoms, whether taken continuously or intermittently (63). Continuous administration is recommended for women with mild symptoms occurring with long-term intervals, as the onset of symptoms cannot be predicted. In woman with serious physical symptoms, continuous dose regimens are more effective than intermittent regimens (60).

2. Luteal phase protocols: This is recommended for women with predictable symptoms that last more than a week before the onset of menstruation (64). The treatment is commenced on the 14th day of the cycle and usually stopped at the beginning of menstruation, but it can continue for a few more days in women with a persistent history of symptoms. This regimen is less expensive and causes fewer side effects. Some individual studies (65,66) and the Cochrane review (46) have reported that SSRIs are equally effective in symptom relief when taken continuously or only in the luteal phase. It has also been reported in some studies that higher doses of SSRI are required in some women receiving luteal phase therapy to adequately treat physical symptoms (65,66).

3. Symptom onset treatment: Intermittent therapy has been shown to be effective from the onset of symptoms to the first days of the month (67). In a randomized study by Yonkers et al. (48), symptom onset therapy with SSRIs was more effective than placebo. Symptom onset therapy is recommended for women with symptoms for a week or less who can easily recognize the onset of their own symptoms (68,69). If intermittent treatments are ineffective or difficult for patients, continuous treatment is recommended (69).

The doses used for PMDD are similar to those used to treat depression. Recommended SSRI doses for SSRI regimens are given in Table 4.

Although the majority of symptomatic women respond to an SSRI, around 30% to 40% may not show any improvement (64). The dose of the SSRI should be adjusted according to the symptoms and lack of response to treatment should not be diagnosed after observing several treatment cycles (64). The possible adverse effects of SSRIs are insomnia, nausea, somnolence, fatigue and reduction in libido (44). Changing the SSRI initiated to a newer agent or switching to luteal phase therapy should be considered in women with side-effects

Table 4. Recommended SSRI doses for premenstrual dysphoric disorder (64)

SSRI	Starting dose (per day)	Usual effective doses* (per day)	Maximum dosage**(per day)
Citalopram	10 mg	20-30 mg	Continous 40 mg Intermittent 30 mg
Escitalopram	5-10 mg	10-20 mg	Continous 20 mg Intermittent 20 mg
Fluxetine	10 mg	20 mg	Continous 30 mg Luteal phase 30 mg Symptom onset 20 mg
Paroxetine	10 mg	20-30 mg	Continous 40 mg Intermittent 30 mg
Sertraline	25 mg	50-150 mg	Continous 200 mg Intermittent 150 mg

*The starting dose can be increased to the usual effective dose if the initial dose is not sufficient to suppress the symptoms,**Maximum dosage required if symptom control is not achieved with the usual effective dose after a number of treatment cycles.
SSRI: Selective serotonin reuptake inhibitors

(69). If the patient fails to show any improvement with more than one SSRI agents, other diseases mimicking PMS/PMDD symptoms, such as PCOS [in which there is a 3.39 times increased risk for depression and a 3.64 times increased risk for anxiety (70)], major depression or substance use disorder must be investigated. In order to avoid withdrawal symptoms, SSRIs if used continuously, should be discontinued gradually. The patients should not continue taking SSRIs prior to and during pregnancy. The optimal duration of the treatment with SSRIs is unknown. The treatment can be continued until women with recurrent symptoms become pregnant or reach menopause (44).

Treatment with combined oral contraceptives

For women with moderate to severe symptoms seeking hormonal contraception, treatment with a COC is recommended, as these medications suppress the hypothalamic-pituitary-ovarian axis and ovulation. Monophasic preparations should be preferred as multiphasic preparations can worsen mood symptoms (44). COC's containing drospirenone, especially with a 24 4+ regimen [3 mg drospirenone (DRSP)/20 mcg ethinyl estradiol] with a shortened drug-free interval of four days are effective and approved by the Federal Drugs Agency for management of PMDD (71). In case of persistence of the PMS/PMDD symptoms and/or presence of intermediate bleeding that does not improve after three month of COC use, the dosage of ethinyl estradiol can be increased and a 21+7 regimen with 3 mg DRSP/30 mcg ethinyl estradiol COC can be commenced. However, the 24/4 regimen is associated with better cycle control (72). The COC can be switched to an alternative formulation if drospirenone-containing COC fails to improve the symptoms or an SSRI can be added to COC monotherapy in order to improve the treatment results. Continuous use of COC is better than intermittent use. In two randomized studies (73,74) and in a meta-analysis (75), it was shown that COCs containing 20 mcg ethinyl estradiol/3 mg drospirenone with a four-day confinement interval are more effective than placebo in reducing PMDD symptoms.

Cognitive behavioral therapy

CBT has been used previously in the treatment of depression and anxiety disorders in women, but data on its use for PMS/PMDD are limited. While some studies report that it is useful (76,77), others failed to show any statistically significant benefit (78). In a randomized study covering 174 women with PMDD, women in the internet-based cognitive behavioral intervention group experienced a decrease in symptom severity compared to a waiting list control group (76). The CBT course may be useful for some women, but data on this are limited and qualified health-service providers are required for delivering this treatment.

Gonadotropin releasing hormone agonists

In women with severe symptoms who cannot respond or tolerate SSRIs or COCs, the next step might be considering GnRH that provides a reversible medical oophorectomy (78). Although GnRH agonist therapy is effective in PMDD (79), it cannot be used for a long period due to the menopausal side-effects related to estrogen deficiency, such as bone loss and genital atrophy. Depot leuprolide acetate 3.75 mg per month is the first choice. Add-back therapy with COC is recommended if GnRH analog will be used longer than six months (44).

Alternative hormonal therapies

Percutaneous estradiol (100 mcg estradiol patches twice weekly) opposed with a cyclical 10-12 day course of oral or vaginal micronized progesterone or long-term progestogen with the LNG-IUS 52 m are recommended regimens for PMS (44). However, careful monitoring is required. Danazol 200 mg twice daily is also effective in reducing the symptoms, although virilizing side-effects including weight gain, acne, hirsutism and deepening of the voice limits its use (44). Effective contraception must be provided during Danazol treatment as Danazol may cause virilization of the female fetus.

Surgery

As medical treatment of PMDD is usually successful, surgery [bilateral oophorectomy/bilateral salpingoophorectomy (surgical menopause)] is considered only in a very few patients who failed to respond to all the medical therapies described (44). Before surgery, all the pharmacological treatment modalities, especially administration of GnRH analogues, should be considered (80,81). In young women HRT must be commenced after bilateral oophorectomy (44).

Conclusion

PMS and PMDD symptoms compromise the well-being of women and have a negative impact on quality of life. Definitive diagnosis is based on prospective self-reporting of the symptoms. Most cases are unrecognized as the presence of the symptoms is not usually questioned during gynecological exams and routine check-ups. Exercise, a healthy diet rich in vitamins and minerals and CBT are the first-line treatment modalities in mild cases. SSRIs and/or COCs are first-line pharmacologic treatments as they are effective in the majority of the women with PMS and PMDD symptoms. Alternative

hormonal therapies can be utilized when the standard therapies fail. Surgery is the last resort and should not be considered until all the alternative medical treatment modalities have been tried and are ineffective.

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Can we use mesenchymal stem cell transplantation for COVID-19 patients in puerperium period?

To the Editor,

The Coronavirus disease-2019 (COVID-19) pandemic has affected the whole world, and pregnant women and women in the postpartum period have not been spared from its effects. Pregnant women during this pandemic pose an unknown risk to their babies. We followed up pregnant and post-pregnancy COVID-19 patients in this period in the intensive care unit (ICU) and used mesenchymal stem cell (MSCs) transplantation in suitable patients. We aimed to investigate patients with COVID-19 pneumonia and who were hospitalized in the ICU after C-section in the puerperium period and either given or not given MSCs treatment.

Case 1: A 33 year-old multiparous woman with a history of pregnancy at 33 gestation weeks, admitted with fever (>38 °C) and cough, with a presumptive diagnosis of COVID-19. Chest computed tomography showed patchy ground-glass opacity (Figure 1). On the third day of hospitalization, she had severe dyspnea and tachypnea and delivery was advised, so she underwent C-section with spinal anesthesia. The patient was transferred to ICU after labor with respiratory distress and X-rays showed patchy ground-glass opacities. She was intubated. D-dimer, C-reactive-protein (CRP), and ferritin levels continued to increase up to the tenth day after the C-section. The patient's clinical situation was evaluated as cytokine storm. After tocilizumab treatment, MSC treatment was given twice with two days intervals. At the end of the third week the patient was extubated. X-rays showed dramatic healing of the patchy ground-glass opacity. When she could breath air unassisted, she was discharged from ICU and subsequently to home.

Case 2: A 34 year-old woman was admitted to ICU after cesarean section with a diagnosis of COVID-19. She had tachypnea and shortness of breath in the postoperative period. High flow nasal cannula oxygenation, intermittent non-invasive ventilation and intermittent prone positioning was applied at ICU follow up. The patient did not respond to seven days of this tretment so she was intubated. Again, D-dimer, CRP, and ferritin levels continued to increase. On the eleventh and twelfth days, she received immune plasma therapy. After 21 days, despite all these interventions, she unfortunately died.

MSCs are tolerated by the recipient immune system while also being immunomodulatory. MSCs are known to function via several mechanisms relevant to acute lung injury. When administered intravenously they sequester in the lung. The effects of MSC are known to include: anti-inflammatory mechanisms; inhibition of lung fibrosis; lung tissue regeneration; and an anti-apoptotic effect in injured cells (1). After MSC injection, cytokine-secreting immune cells, including NK cells, were markedly reduced and pro-inflammatory cytokine tumor

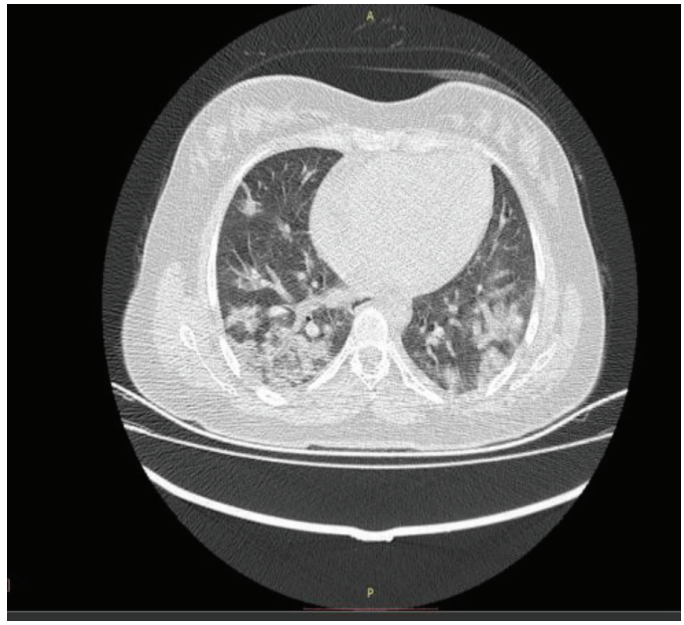

Figure 1. Thorax CT image before ICU admission
CT: Computed tomography, ICU: Intensive care unit

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necrosis factor-alpha was decreased (2). MSC transplantation may be an appropriate choice of therapy in patients with severe COVID-19 to prevent severe morbidity and mortality in critically ill patients in the future.

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Sexual function, depression, anxiety, and vulvovaginal candidiasis

To the Editor,

We read the paper by Moshfeghy et al. (1) entitled "association of sexual function and psychological symptoms including depression, anxiety, and stress in women with recurrent vulvovaginal candidiasis" published in June 2020, volume 21, issue 2 of your journal with great interest. Female sexuality is a highly complex and multifactorial issue. The effect of the vaginitis that every woman experiences "at least once" in her lifetime, especially candidal, on sexual function cannot be underestimated. The authors aimed to make an objective assessment, as far as possible, using the Female Sexual Function Index (FSFI) the most commonly used questionnaire in the world for this assessment. However, in our opinion determining some other variables while evaluating the problem could strengthen the study. In addition, when examining the regression analysis, it is unclear if this was univariate or multivariate and, therefore, the relationship of the variables with the subject is not revealed. We would like to highlight three issues on this subject. Firstly, and most importantly, the demographic characteristics of the patients have not been presented. The characteristics of the study and control groups such as age, body mass index (BMI), occupation, education level, and substance addiction were not given. Especially, age affects sexual functions concerning BMI body perception. The second important issue was the respective "male sexual" function. According to the "Global Study of Sexual Attitudes and Behaviors", 28% of sexually active men in the general population have at least one sexual problem (2). Periodic to frequent early ejaculation was reported by 14% of men, slightly more frequently than erection difficulties (10%), and a total of 9% complained of lack of interest in sex (2). These male dysfunctions will clearly also affect female sexual function (3). In all societies, especially in developing countries, the effect of male sexual dysfunctions on women is overlooked. It is acceptable that this study did not include an evaluation of male sexual function, but it might be appropriate to mention it as an important limitation. The third and last issue is that conditions such as polycystic ovary syndrome (PCOS), endometriosis, pelvic masses, and urinary incontinence, which can cause psychological and sexual dysfunction in women, have not been excluded. For instance, although different results were reported for sexual dysfunction in PCOS patients, it was stated that depression and anxiety are more common in these patients and, in evaluations made with FSFI, there are often variations in satisfaction scores, especially concerning hirsutism and BMI (4,5). Considering the results reported by the authors, mentioning these factors, which have been reported to have an effect on depression, anxiety, and sexual function, would provide a clearer evaluation of the findings of the study for us readers.

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Author's Response

Dear Editor,

We would like to thank Başer et al. (1) for their interest in our paper and for taking the time to express their concerns. In their letter to the editor, they noted some points that need to be clarified by the authors (1). First, although the demographic characteristics of the patients did not report as a table in the results but we assessed the characteristics including age, education, occupation status and body mass index in case and control group and there was no significant relationship between demographic characteristics in two groups (Table 1). Second, we agree with the probable effect of male sexual dysfunction on female sexual function and the issue could be one of our study limitation. Third, in the method of our article, we mentioned that all individuals in case group were married women with only a history of at least four episodes of vulvo-vaginal candidiasis per year according to documented diagnosis of symptomatic episodes of infection in their clinic records (2). They were not known cases of other gynecologic problems such as polycystic ovary syndrome, endometriosis, pelvic masses, and urinary incontinence because according to mentioned method, we assessed each participant' health information in sample recruitment process and these women who had these problems were not included in the study. Also, this issue was stated in our article about the control group consisted of healthy individuals who were referred to clinics for routine screening. Therefore, we tried to select case and control groups due to difference in history of recurrent vulvovaginal candidiasis.

Zeinab Moshfeghy[1], Somayeh Tahari[2], Roksana Janghorban[3], Fatemeh Sadat Najib[4], Arash Mani[5], Mehrab Sayadi[6]

Table 1. Comparison demographic characteristic between the case and control groups

Variable	Groups		p*
	Case	Control	
Age (mean ± SD)	32.80±7.64	31.82±7.01	0.506
Education (n, %)			
Illiterate	0	2 (4)	0.595
Primary school	9 (18)	7 (14)	
Secondary school	11 (22)	9 (18)	
High school	4 (8)	5 (10)	
High school diploma	10 (20)	15 (30)	
University degree	16 (32)	12 (24)	
Occupation (n, %)			
Housewife	43 (86)	42 (84)	0.424
Employee	7 (14)	8 (16)	
Monthly income (n, %)			
Less than 20.000.000 IRR	7 (14)	7 (14)	0.424
20.000.000-40.000.000 IRR	36 (72)	32 (64)	
More than 40.000.000 IRR	7 (14)	11 (22)	
BMI (kg/m^2) (mean ± SD)	24.8±2.6	25.2±2.5	0.134

*P<0.05 was considered statistically significant; t-test and chi-square were used for variables.
SD: Standard deviation, IRR: Iranian rial, BMI: Body mass index

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Laparoscopic removal of the cervical stump for a cervical solid mass in a patient with previous supracervical hysterectomy

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Abstract

The aim of this video article is to demonstrate a surgical tutorial in which a cervical mass was resected with laparoscopic removal of the cervical stump after supracervical hysterectomy (SCH). First, the dense adhesions due to previous operation were dissected. Then the dissection was continued carefully in order to identify bilateral ureters and iliac arteries. A cervical mass of nearly 4 cm was identified at the posterosuperior aspect. The mass was separated from surrounding tissue and removal of the cervical stump was performed. This is an exciting case, illustrating that minimally invasive procedures, such as laparoscopic removal of the cervical stump after SCH, can be used in the management of benign cervical tumor. The main feature of this operation was to check the relationship of tumor with adjacent structures, including ureters, bowel and vascular structures at every step to prevent unwanted injuries.

Keywords: Cervical mass, laparoscopy, cervical stump

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Introduction

Laparoscopic removal of the cervical stump was first reported by Nezhat et al. (1). It can be considered a definitive therapy for patients with recurrent symptoms after supracervical hysterectomy (SCH) (2). There are some significant risk factors, including younger age, pelvic pain and endometriosis, for removal of the cervical stump in patients who have previously undergone SCH (3). It can be performed vaginally, abdominally or laparoscopically and either with or without robotic assistance. If a laparoscopic approach is chosen, advanced experience on laparoscopy is crucial (1). Here, we present a surgical tutorial in which a cervical mass was resected, with laparoscopic removal of the cervical stump (Video 1).

A 50-year-old G2P1 woman was referred to our hospital with the complaint of pelvic pain for three months. She had a history of SCH for abnormal uterine bleeding due to myoma uteri 10 years previously. Bimanual pelvic examination revealed tenderness and vaginal speculum examination was normal. A solid mass of nearly 4x4 cm was identified at the cervical stump on transvaginal ultrasound. Laboratory tests for tumor biomarkers were negative while magnetic resonance imaging revealed a 4 cm in diameter solid mass localized at the cervical region. The patient underwent gastroscopy and colonoscopy because of malignancy suspicion but no abnormality was detected. Endocervical biopsy and Pap smear results revealed no abnormality. After fully informing the patient, consent was given for the use of images and videos of the procedure.

After the patient preparation, a uterine manipulator was inserted vaginally with a colpotomizer. A 10 mm umbilical port and three 5 mm abdominal ports were placed and the pelvis was explored. There were extremely dense adhesions between bowel, omentum and the anterior abdominal wall. First, the dense adhesions, due to the previous operation, were dissected. Then the dissection was carefully continued in order to identify bilateral ureters and iliac arteries (Figure 1). The

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previously identified 4 cm cervical mass at the posterosuperior aspect was identified (Figure 2). The mass was separated from surrounding tissue and a laparoscopic removal of the cervical stump was performed. After this procedure, the bladder was investigated with methilene blue for any signs of injury. The operation time was 185 minutes. There were no complications. The postoperative course was uneventful and the patient was discharged on the third postoperative day. Histopathological examination confirmed a benign, 4 cm leiomyoma.

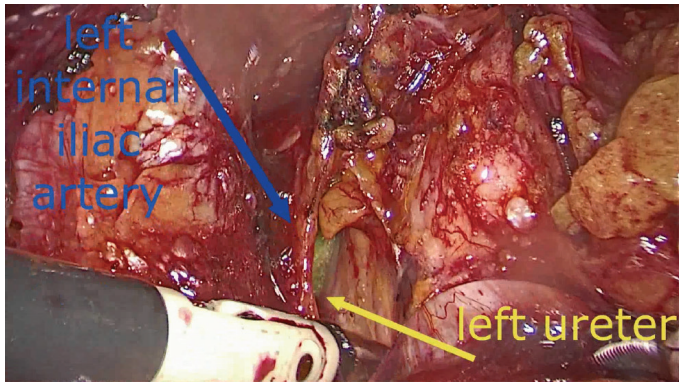

Figure 1. Appearance of left ureter and left internal iliac artery

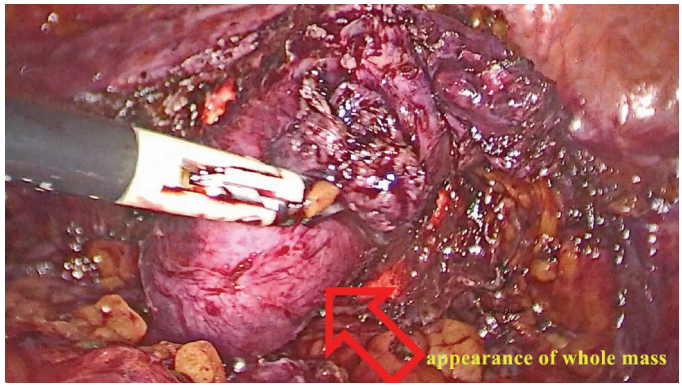

Figure 2. Appearance of the whole mass

This is an exciting case illustrating that minimally invasive procedures, such as laparoscopic removal of the cervical stump after SCH, can be used in the management of benign cervical tumor. The main feature of this operation was to check the relationship of tumor with adjacent structures, including ureters, bowel and vascular structures at every step to prevent unwanted injuries.

Video 1.

https://www.doi.org/10.4274/jtgga.galenos.2019.2019.0143.video1

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

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Inguinofemoral lymphadenectomy and femoral dissection: cadaveric educational video

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Abstract

Vulvar cancer is rare. The complex inguinofemoral anatomy and the limited number of surgical procedures per year per gynecological oncologist tends to decrease the competency level. This step-by-step, cadaveric educational video was produced to increase understanding of the anatomy and technique of inguinofemoral lymphadenectomy.

Keywords: Inguinofemoral, lymphadenectomy, vulvar cancer, dissection, groin

Received: 04 February, 2019 **Accepted:** 26 March, 2019

Introduction

Since vulvar cancer is a rarely seen gynecological malignancy, every year only a limited number of surgeries are likely to be performed by each gynecological oncologist. Van der Zee et al. (1) suggested that at least 5 to 10 patients should be managed per year by each surgeon to ensure the competency level. Additionally, adoption of a sentinel lymph node approach has led to a less radical surgical concept that further reduces the number of cases with full inguinofemoral lymphadenectomy. The current situation has motivated gynecological oncology fellows and young surgeons to gain education and practice by cadaveric dissections. This cadaveric surgical educational video demonstrates the technique of inguinofemoral lymph node dissection in a step-by-step process.

Material and Methods

This cadaveric educational dissection video was recorded at Hacettepe University Faculty of Medicine, Department of Anatomy. One limitation of this study was that a fresh frozen cadaver was not used. However, the video was recorded to demonstrate a step-by-step technique and we believe one of the strong points of this study is that, whether the cadaver is fresh frozen or not, if used properly this type of study still has an important place in medical and post-graduate education.

Inguinal anatomy

The groin is the anatomical region between the anterolateral abdominal wall and lower extremity. The inguinal ligament, which is one edge of the femoral (Scarpa's) triangle, lies between the anterior superior iliac spine and pubic tubercle. The inguinal ligament is formed by the aponeurosis of the external oblique muscle. Under the skin the superficial fascia may be observed and it is composed of a fatty superficial layer (Camper's fascia) and a deeper membranous layer (Scarpa's fascia). Superficial vessels, which supply the lateral, middle and medial sites are the superficial circumflex iliac, superficial epigastric and superficial external pudendal vessels, respectively. Scarpa's fascia fuses with the fascia lata, the deep fascia of the thigh. Under the fascia lata the femoral vessels and nerve can be dissected. They lie as nerve-artery-vein-lymphatics formation from lateral to medial. The femoral

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vessels are covered by the femoral sheath, which is the continuation of transversalis and iliac fascia, below the inguinal ligament. Additionally, the femoral triangle is detected under the fascia lata. The lateral border is the sartorius muscle and the medial border is the adductor longus muscle. The fossa ovalis is approximately 3 cm inferolateral to the pubic tubercle and it is an opening through the fascia lata where the great saphenous vein enters to the femoral vein. This part is covered by a thin, multi-perforated fascia called the fascia cribrosa, and many lymphatics and venous structures pass through this region (Figure 1) (2,3).

Inguinal lymph nodes

The inguinal lymph nodes are categorized into the superficial and the deep group, separated by the fascia lata. The superficial lymph nodes are located under Scarpa's fascia and divided into five groups, depending on the termination point of the great saphenous vein; superomedial, superolateral, inferomedial, inferolateral and central (4). The deep inguinal lymph nodes are located beneath the fascia lata, medial to the femoral vein (5). The lymph node found at the femoral canal, anterosuperior to the femoral vein, is known as Cloquet's node, the nexus between the deep inguinal and iliac/obturator lymph nodes (6).

Surgical procedure

This surgical procedure is performed in the low lithotomy position, at the left groin. Between the anterior superior iliac spine and pubic tubercle the skin incision is performed, 8 cm in length, and 2 cm below and parallel to the inguinal ligament. After the incision, the dissection deepens to identify Camper's fascia lying over Scarpa's fascia. Identification and preservation of Camper's fascia is critical in live patients to secure the skin flap and prevent skin necrosis. To dissect the fibrofatty tissue containing the superficial inguinal lymph nodes between the Scarpa's fascia and the fascia lata, the first step should be to mobilize the fibrofatty tissue under Scarpa's fascia and then dissect 2 cm cephalad, to the inguinal ligament where the aponeurosis of the external oblique muscle is seen. Afterwards, excision of the lymphatic and fibrofatty tissue is performed from lateral (superficial circumflex iliac vein) to medial (superficial external pudendal vein) and superior to inferior, identified by the inferomedial end of inguinal ligament where it intersects with the adductor longus muscle.

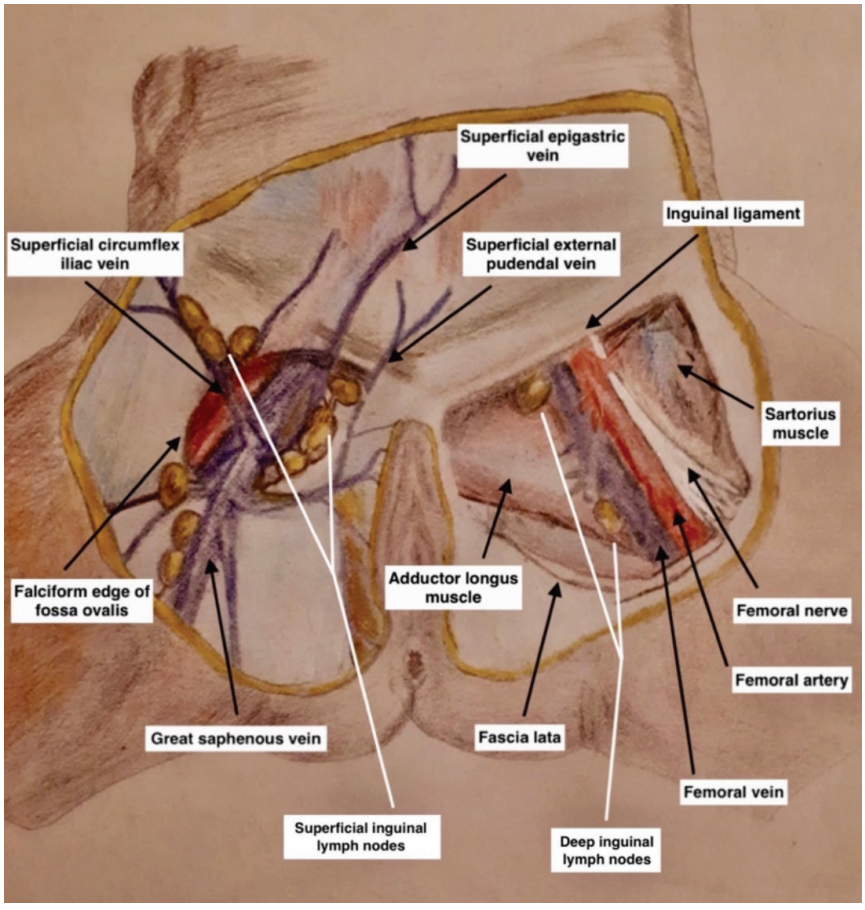

Figure 1. Inguinal anatomy with regard to superficial and deep inguinal lymph nodes (the figure was illustrated from the book "Atlas of Human Anatomy, Pelvis and Perineum, Plate 389, 7th Edition, 2019 Elsevier, Netter Frank H." by Sedef Yasin

The fossa ovalis is encountered after resection of superficial inguinal lymph nodes (Figure 2). Medial to the falciform edge of the fossa ovalis, dissection of the cribriform fascia will lead to the deep inguinal lymph nodes, which are located medial to the femoral vein. The great saphenous vein enters the femoral vein from the opening of the fossa ovalis, so careful dissection is essential while removing the cribriform fascia. Lymphadenectomy is performed towards the apex of the femoral triangle, and there is no need to dissect the femoral sheath to excise the deep inguinal nodes completely (Figure 3). Finally, Cloquet's lymph node, the most superior of the deep inguinal nodes, may be resected from the femoral canal under the level of the inguinal ligament (7).

Complications

Hematoma, seroma, wound breakdown, wound infection and lymphedema are the most probable complications after inguinofemoral lymphadenectomy. The most serious complication is lymphedema and the great saphenous vein should be secured during dissection to decrease the risk of lymphedema (8).

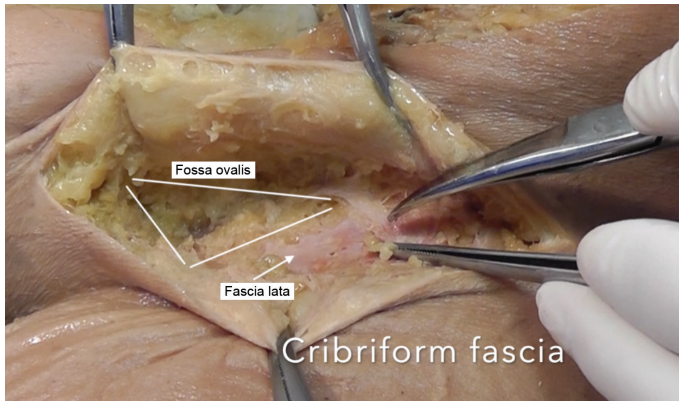

Figure 2. Cribriform fascia over the fossa ovalis, and fascia lata, after superficial inguinal lymphadenectomy

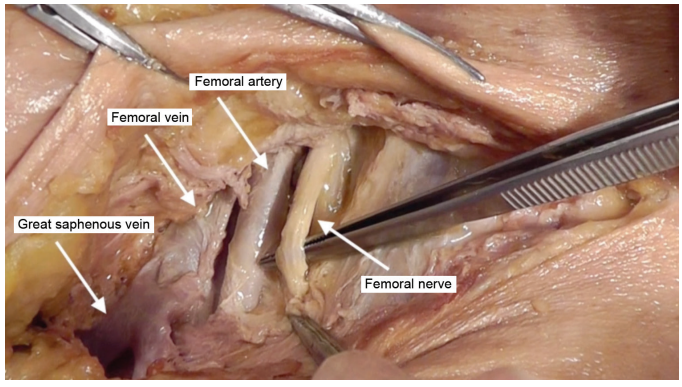

Figure 3. Femoral nerve, artery, vein and great saphenous vein after dissection of the femoral sheath

Video 1.

https://www.doi.org/10.4274/jtgga.galenos.2019.2019.0026.video1

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Laparoscopic and imaging findings of growing teratoma syndrome

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Abstract

Growing teratoma syndrome (GTS) occurs during post-treatment observation of ovarian malignant germ cell tumors. The characteristic features of this syndrome are normal tumor marker levels and evident tumoral mass or implants on imaging studies. We report a case of GTS in a 22-year-old woman with a malignant germ cell tumor. After staging surgery and chemotherapy, computed tomography showed peritoneal implants. Laparoscopy was planned to exclude malignant recurrence. During laparoscopy, smooth and pink-colored lesions were seen at the lateral pelvic wall. Histopathologic evaluation reported mature teratoma tissue with extensive mature glial components. GTS is not a malignant condition and the benefit of radical surgical intervention in cases without mass-related complication is not proven. GTS should be kept in mind after primary treatment of ovarian immature teratoma.

Keywords: Growing teratoma syndrome, laparoscopy, management

Received: 21 February, 2019 **Accepted:** 27 April, 2020

Introduction

A 19 year-old nulli-gravid woman with massive ascites and abdominal mass was referred to our clinic. Laboratory examination revealed elevated tumor markers (AFP: 922.4 ng/mL, CA 125=331 U/mL, CA 19-9=418.1 U/mL). Computed tomography (CT) revealed a huge mass filling the abdomen and a large volume of ascites (Figure 1). Midline laparotomy was performed due to the pre-diagnosis of ovarian malignant germ cell tumor. During the intraoperative observation, a huge left-side ovarian mass and massive peritoneal-omental implants were detected. Subsequently, left salphingo-oophorectomy, total omentectomy, peritonectomy, and bilateral pelvic and para-aortic lymph node dissection were performed. There was no residual tumor at the end of the surgery. Final pathology was stage IIIC immature teratoma with retroperitoneal lymph node involvement. Four cycles of bleomycine-etoposide-paclitaxel chemotherapy was administered. At the end of the treatment tumor markers were within normal range.

Two months after chemotherapy and six months after the operation, during a routine follow-up visit, CT examination revealed peritoneal implants. Tumor markers remained within normal ranges and the patient was asymptomatic. Recurrence of malignant germ cell tumor was not expected and so

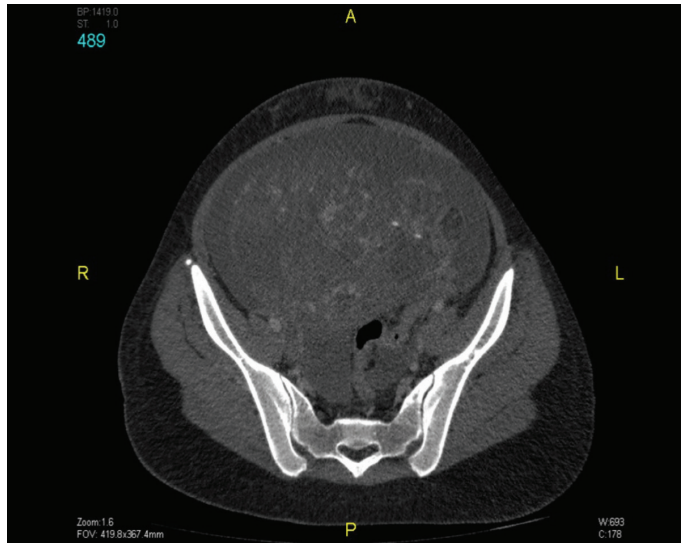

Figure 1. Preoperative computerized tomography image of the abdominal mass

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laparoscopy for further differential diagnosis was scheduled.

During laparoscopy, smooth and pink-colored lesions were detected at the lateral pelvic wall (Video 1). These lesions were superficial and easily removable (Figure 2). There was no invasion into the sub-tissue. The operation was completed after taking multiple biopsies from the lesions detected.

There were no postoperative complications. Histopathological evaluation revealed mature teratoma tissue with extensive mature glial components. The diagnosis was growing teratoma syndrome (GTS) and adjuvant therapy was considered unnecessary. The patient is alive without recurrence since the initial diagnosis of germ cell tumor 15 months earlier and is followed up every three months. Information consent was given by the patient for this report.

Discussion

GTS is characterized by the presence of tumoral lesions, growing during or after chemotherapy being given for malignant germ cell tumors, and when tumor markers are within normal range. It is a rare condition and is more common in men with testicular germ cell tumors than in women with ovarian germ cell tumors (1). The incidence of GTS is unknown. The median (range) interval between the diagnosis of ovarian immature teratoma and GTS has been reported to be nine (4-55) months (2). Although approximately 100 cases have been reported in the English literature, a recent article found that GTS is more common than previously reported, with an incidence of 40% in patients who had already undergone surgery (3,4). Mostly, it occurs in the pelvic region but there are also some patients with extra-pelvic disease, such as lung, mediastinum, central nervous system, spleen and diaphragm (5-7).

The benefit of radical surgical intervention in cases without mass-related complication has not been proven. Disease can be asymptomatic and totally stable over a long period, which raises the question of a more conservative surgical approach in patients with massive peritoneal spread (8). Radical surgeries, such as peritonectomy, splenectomy or bowel resection may be needed to achieve complete debulking (9). However, these radical interventions are not standard in asymptomatic GTS (3). Radical surgery should be performed in symptomatic patients as a final resort.

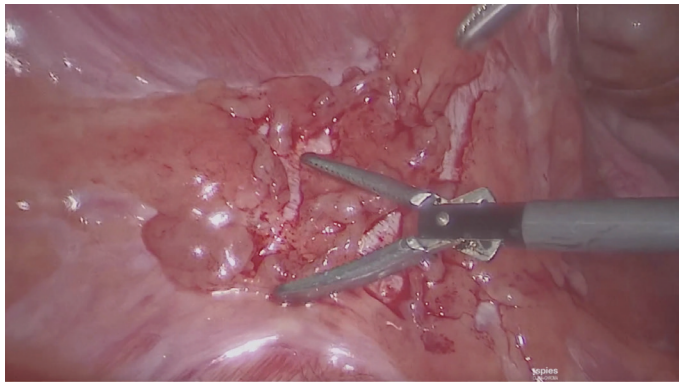

Figure 2. Laparoscopic image of the peritoneal implants

As has been noted, this condition can be managed by observation without any therapeutic intervention. However, GTS should be definitively diagnosed and malignant germ cell tumor recurrence should be excluded prior to a final management decision. Imaging studies may not be sufficient to make the differential diagnosis between malignant recurrence and GTS. Laparoscopy is thus helpful to distinguish GTS from malignant recurrence and to preclude unnecessary chemotherapy. In addition, laparoscopy can be helpful to observe the abdomen directly and to obtain tissue for a definitive diagnosis.

Video 1. Laparoscopic findings of peritoneal implants

https://www.doi.org/10.4274/jtgga.galenos.2020.2019.0034.video1

Informed Consent: *Information consent was given by the patient for this report.*

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

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

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

June 04-06, 2021	**Twins Congress, Beijing, China**
June 23-26, 2021	**International Urogynecological Association 46th Annual Meeting, Singapore**
June 24-26, 2021	**VII. MIPS Annual Meeting, Athens, Greece**
June 27-30, 2021	**European Society of Human Reproduction and Embryology (ESHRE) virtual 37th Annual Meeting (VIRTUAL)**
July 06-09, 2021	**Society for Reproductive Investigation 68th Annual Meeting, Boston, United States**
July 14-17, 2021	**XXVII European Congress of Perinatal Medicine (ECPM), Lisbon, Portugal**
August 30-Sep 02, 2021	**International Gynecologic Cancer Society (IGCS) Meeting, Rome, Italy**
October 03-05, 2021	**ESGE 30th Annual Congress, Rome, Italy, onsite + virtual**
October 16-20, 2021	**American Society for Reproductive Medicine (ASRM) 77th Annual Meeting, Baltimore, Maryland, United States**
October 23-25, 2021	**22nd European Congress on Gynaecological Oncology, Prague, Czech Republic + Online**
November 14-18, 2021	**50th American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Austin, Texas, United States**

CONGRESS CALENDER

NATIONAL MEETINGS

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

June 11-13, 2021	**Fetal Tıp ve Prenatal Tanı Kongresi 2021, Ankara**
September 10-12, 2021	**9. Acıbadem Kadın Doğum Günleri, İstanbul**
September 23-26, 2021	**3. Obstetrik ve Jinekoloji Tartışmalı Konular Kongresi, KKTC**
October 01-03, 2021	**39. Zeynep Kamil Jineko-Patoloji Kongresi, Online**
October 13-16, 2021	**4. Uluslararası Kozmetoloji ve Kozmetik Jinekoloji Kongresi, İstanbul**
October 28-31, 2021	**Türkiye Maternal Fetal Tıp ve Perinatoloji Derneği Ultrasonografi Kongresi, İstanbul**
October 28-31, 2021	**8. Üreme Tıbbı ve Cerrahisi Derneği Kongresi, Antalya**
November 10-14, 2021	**9. Üreme Sağlığı ve İnfertilite Kongresi – TSRM, Antalya**
December 01-05, 2021	**18. Ulusal Jinekoloji ve Obstetrik Kongresi, Antalya**
December 02-05, 2021	**İstanbul Üniversitesi 10. Kadın Doğum Günleri, İstanbul**
December 03-05, 2021	**Çukurova Kadın Doğum Günleri 2021, Adana**
December 09-12, 2021	**4. Karadeniz Jinekoloji ve Obstetrik Kongresi, Online**