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Clinical Investigations

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galenos yayınevi

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

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

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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- A short title of no more than 50 characters, including spaces, for use as a running foot.

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

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

Precis

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

'Using a 45 point questionnaire, we have evaluated the trend of Robotic surgery training in the gynecologic surgery fellowship programs across the nation'.

Abstract

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

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

· Materials and Methods: Study design, participants, outcome measures, and in the case of a negative study, statistical power.

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

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

Authors from Turkey or Turkish speaking countries are expected to submit a Turkish abstract including subheadings such as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç". The abstract of Authors whose native language is not Turkish will be provided free of charge translation services into Turkish language.

A structured abstract is not required with review articles and case reports.

Keywords

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

Turkish abstracts should have keywords "Anahtar Kelimeler" picked from www.atifdizini.com under "Türkiye Bilim Terimleri" link.

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

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

Original researches should have the following sections;

Introduction

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

Materials and Methods

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

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

Results

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

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

Discussion

Begin with a description of what your study found in relation to the purpose or objectives as stated in the Introduction. State the importance and significance of your findings to clinicians and actual patient care but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with previous studies with explanations in cases where they differ, although a complete review of the literature is not necessary.

Study Limitations

Provide information on the limitations of the study. No new data are to be presented in this section. A final summary is not necessary, as this information should be provided in the abstract and the first paragraph of the Discussion. Although topics that require future research can be mentioned, it is unnecessary to state, "Further research is needed."

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

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The main text of case reports should be structured with the following subheadings:

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LETTER FROM THE PRESIDENT

Dear Colleagues,

As we celebrate the new year, I wish everyone a healthy, happy, peaceful and successful year. I hope that it will be a year full of collaboration and science.

I would like to especially thank the obstetrics and gynecology community who support our journal through submitting their articles and researches. We progress further with your contributions and comments.

It is such an honor to announce the most prosperous and the greatest congress in terms of the scientific and the social programme, in the field of obstetrics and gynecology, the 18th National Gynecology and Obstetrics Congress, which will be held between April 18-22, 2020, in Kaya Palazzo Golf Resort, Antalya. I would like to welcome all our colleagues at our congress. I think that it would be a great opportunity to share and discuss the latest updates in our field together. Our congress would become more meaningful and fulfill its aim with your participation. Therefore, we are looking forward to receiving your scientific studies including papers, articles, and posters, which will be submitted to the congress.

We will be glad to be with you in such a great atmosphere in Antalya.

Best regards,

Ateş Karateke, Prof. MD.,
President of TSOG

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

I would like to emphasize a significant issue, the increase in the number of residents of obstetrics and gynecology. The number of residents of obstetrics and gynecology has been rapidly increased by the health authorities. With the civil society organizations, we were not involved in the determination of such an increase.

The rapid increase in the number of residents raises concerns regarding the quality and the standardization of resident education. With double the number of residents, the problem in both standardization and the quality of resident education emerge. We have to take into consideration that the education of residents is actually what creates the future of obstetrics and gynecology.

I believe that we, as the community of obstetrics and gynecology, could overcome this issue with collaboration and cooperation among all obstetricians and gynecologists.

Sincerely

Eray Calışkan
Editor in Chief

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2019;16:213-8

Induction of labor with oxytocin in pregnancy with low-risk heart disease: A randomized controlled trial

Düşük riskli kalp hastalığı olan gebelerde oksitosin ile doğum indüksiyonu: Randomize kontrollü bir çalışma

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Abstract

Objective: To compare maternal and perinatal outcomes in pregnant women with underlying heart disease who underwent induction of labor with those who had spontaneous labor.

Materials and Methods: A total of 50 pregnant women with heart disease who were registered in cardio-obstetric clinic were recruited consecutively between 38-41 weeks' gestation. Patients with favorable Bishop scores at 38 weeks were randomized into two groups. Induction of labor with oxytocin was performed in one group, and the second group underwent spontaneous onset of labor. Descriptive analysis in terms of mean, standard deviation, and percentage was performed. Unpaired t-test was applied for comparison of two groups using SPPS statistical software.

Results: No significant difference in the rate of maternal complications was observed between the two groups. No cardiac complications were reported in pregnant females who underwent induction of labor. Fifty-two percent of patients delivered during workday hours when labor was induced, whereas only 24% of pregnant women delivered during working hours who underwent spontaneous delivery. No maternal or neonatal deaths were reported.

Conclusion: Induction of labor with oxytocin is a relatively safe procedure in women with heart disease, it does not result in any cardiac complications. More patients delivered during daytime when electively induced, which minimized the maternal and fetal risks because obstetric, anesthesiologist, cardiologist, and perinatologist specialists are readily available during the daytime.

Keywords: Heart disease, labor, induction, caesarean, delivery

Öz

Amaç: Altta yatan kalp hastalığı olup, doğum indüksiyonu geçirenlerde ve spontan doğum yapan gebelerde maternal ve perinatal çıktıları karşılaştırmaktır.

Gereç ve Yöntemler: Kardiyo-obstetrik kliniğine kayıtlı, kalp hastalığı olan, 38-41 hafta arası gebelik dönemindeki toplam 50 kadın art arda değerlendirildi. Otuz sekizinci haftada Bishop skorları uygun olan hastalar iki gruba randomize edildi. Birinci grupta oksitosin ile doğum indüksiyonu gerçekleştirilirken, ikinci gruba spontan başlangıçlı doğum yapıldı. Ortalama, standart sapma ve yüzde cinsinden tanımlayıcı analiz yapıldı. SPSS istatistik yazılımı kullanılarak iki grubun karşılaştırılmasında eşleşmemiş t-testi uygulandı.

Bulgular: İki grup arasında maternal komplikasyon oranlarında anlamlı farklılık gözlenmemiştir. Doğum indüksiyonu yapılan gebe kadınlarda kardiyak komplikasyonlar bildirilmemiştir. Hastaların %52'si doğum indüklendiğinde çalışma saatleri içerisinde doğum yaparken, spontan doğum yapan gebe kadınların sadece %24'ü çalışma saatleri içerisinde doğum yapmıştır. Maternal veya neonatal ölüm rapor edilmemiştir.

Sonuç: Oksitosin ile doğum indüksiyonu, kalp hastalığı olan kadınlarda oldukça güvenli olup, herhangi bir kardiyak komplikasyona neden olmamaktadır. İsteğe bağlı olarak indüklendiğinde gündüz daha fazla hasta doğum yapmış olup; obstetrik, anesteziyolojist, kardiyolog ve perinatolog uzmanlarının gündüzleri kolaylıkla hazır bulunması maternal ve fetal riskleri en aza indirmiştir.

Anahtar Kelimeler: Kalp hastalığı, doğum sancısı, indüksiyon, sezaryen, doğum

PRECIS: Elective induction of labor with oxytocin is a relatively safe procedure in women with low-risk heart disease.

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Introduction

About 1% of all pregnancies are complicated by cardiovascular disease, and the incidence is perpetually rising with the advancement of the medical field, as raised treatment standards allow more patients with heart disease to reach childbearing age[1,2]. As is evident, during pregnancy, labor, delivery, and the postpartum period, hemodynamic alterations are bound to occur[3], the odds of maternal mortality and morbidity surge considerably with a compromised heart[4], heart disease, which complicates pregnancy, may be either congenital or acquired. Cyanotic heart diseases, diminished systemic ventricular function, complex congenital heart disease, left ventricular outflow tract obstruction, pulmonary hypertension, or mechanical valves are at maximum risk of developing complications during pregnancy[5]. Maternal mortality varies directly with the functional class of heart disease: 0.4% for New York Heart Association (NYHA) classes I and II, and 6.8% for classes III and IV. The maternal functional class also influence the fetal mortality, varying from zero for class I to 30% for class IV[6,7]. The management of delivery in such cases is vital to the survival of both mother and baby and should be performed under the presence of a team of obstetricians, cardiologists, anesthetists, and perinatologists. Several studies have authenticated that cesarean section is accomplished more often in women with heart disease than in the healthy population[8,9]. However, the cesarean section is associated with more blood loss and higher thromboembolic and infection risks; therefore, vaginal delivery is favored in such cases, and cesarean section is reserved for obstetric indications[10]. Vaginal delivery with low-dose regional analgesia and careful fluid management is the preferred mode of delivery in most cases[11].

Vaginal deliveries can either be spontaneous or induced. An upsurge has been observed in the rate of labor induction among women with term pregnancies during the past 10-15 years[12,13]. However, labor must be induced with caution in women with heart disease because the physiologic rise in cardiac output may burden an already compromised heart[14]. It has been observed that majority of the hospital deliveries occurring during the night hours are associated with adverse perinatal outcomes[15]. The rates of emergency caesarean sections and neonatal intensive care unit (NICU) admissions are also increased considerably in the night-shift period[16]. Labor induction for pregnant women with underlying heart disease is often offered to control the timing and setting of labor, to ensure the presence of a team of cardiologists, obstetricians, anesthetists, and perinatologists[17,18].

There is limited literature comparing induction of labor and spontaneous labor in such a group of patients. So far, the induction of labor has been performed for obstetric indications only, and not the heart disease per se. The aim of this prospective randomized controlled study was to compare maternal and perinatal outcomes in these two groups, which might help in formulating guidelines regarding the management of these patients and controlling the timing of delivery in these patients, to avoid night deliveries.

Materials and Methods

The present prospective randomized controlled trial was conducted in the Department of Obstetrics and Gynecology at a tertiary care referral and research institute of Northern India. Clearance from the institute's Ethical Committee was obtained for the study and was performed following ethical standards. A total of 50 pregnant women with heart disease who were registered in the cardio-obstetric clinic were recruited consecutively between 38-41 weeks' gestation after obtaining informed consent. Randomization was performed using a computer-generated random number tables in a 1:1 ratio and sealed opaque envelopes. Women with a known case of heart disease with NYHA class I-II and cephalic singleton gestation were included in the study. Patients with previous cesarean section, primary pulmonary hypertension, Eisenmenger syndrome, Marfan syndrome, left heart obstruction, prior cardiac event or arrhythmia, malformed fetus, severe anemia (<7 g/dL), intrauterine fetal death, other obstetric indications for induction of labor, and patients on anticoagulation were excluded from the study. The cervical assessment was performed using Bishop[19] scores at 38 weeks. A sealed envelope was opened if a score of more than or equal to 6 was found and patients were allocated to the assigned group and admitted electively. Patients with a score of less than six were reassessed after one week till 41 weeks, and randomization was performed when the Bishop score was more than or equal to 6. The two groups were as follows:

Table 1. Distribution of pregnant women with cardiac disease

	Group I (n=25)	Group II (n=25)	p value
Gravidity			
Primigravida	7 (28%)	9 (36%)	0.543
Multigravida	18 (72%)	16 (64%)	0.543
Gestational age at delivery (weeks)	19 (76%)	20 (80%)	0.651
38-39 weeks	5 (20%)	3 (12%)	0.651
39.1-40 weeks	1 (4%)	2 (8%)	0.651
40.1-41weeks	38.5	38.5	0.687
Mean gestational age (weeks)	(0.735)	(0.657)	
Type of cardiac disease			
Congenital heart disease	7 (28.0%)	5 (20.0%)	0.741
Rheumatic heart disease	16 (64.0%)	17 (68.0%)	0.764
Mitral valve prolapse with mitral regurgitation	2 (8.0%)	3 (12.0%)	0.999
Cardiac status			
NYHA class I	24 (96%)	24 (96%)	0.999
NYHA class II	1 (4%)	1 (4%)	0.999
Prior cardiac surgery	3 (12%)	6 (24%)	0.463

NYHA: New York Heart Association

Group I: Induction of labor was started in the morning with oxytocin. An infusion of 30 U oxytocin diluted in 500 mL normal saline was prepared and given through an infusion pump at an initial rate of 3 mU/min. Subsequently, the dose was increased by 3 mU/min every 45 min till adequate uterine contractions were established (at least 3-5 uterine contractions every 10 minutes).

Group II: Patients waited for spontaneous onset of labor.

In both groups, progress of labor was monitored similarly. Multidisciplinary care from cardiologists, obstetricians, and anesthetists was provided to all patients admitted to the labor room. Epidural analgesia was provided wherever feasible, or an injection of morphine 2-5 mg was given intravenously. Epidural analgesia was provided using 0.0625% bupivacaine with a 25 microgram fentanyl bolus of 6-8 mL, followed by infusion of 0.1% bupivacaine with two microgram/mL fentanyl at 6-8 mL/hr. Infective endocarditis prophylaxis was given before epidural analgesia, at spontaneous rupture of membranes or when the delivery was imminent. The second stage of labor was cut short wherever needed. The third stage of labor was actively managed. Patients were observed for postpartum hemorrhage and infections in the immediate postpartum period.

The outcome measures were: obstetric outcome in terms of duration of labor, mode of delivery, and rate of cesarean section with indication; maternal outcomes in terms of postpartum hemorrhage, infection, development of cardiac complications such as pulmonary edema, heart failure or pulmonary embolism, and maternal death; and neonatal outcomes in terms of apgar score, need for admission to the NICU, and neonatal death. Descriptive analysis in terms of mean, standard deviation and percentage was performed. The statistical analysis was conducted using the chi-square and Fisher's exact test for categorical data. The unpaired t-test was used for the comparison of two groups because the data were normally distributed. All statistical tests were two-sided and performed at a significance level of α=0.05.

Results

The study group included 50 pregnant women with documented cardiac disease, of whom 25 underwent induction of labor (group I) and 25 had the spontaneous onset of labor (group II). The distribution of pregnant women in terms of gravida, gestational age of delivery, and type of preexisting cardiac disease is depicted in Table 1. The majority had a gestational age of 38-39 weeks at delivery, with mean gestational age 38.5±0.735 weeks in group I and 38.5±0.657 weeks in group II. Ninety-six percent of patients in each group were of NYHA class I type of cardiac disease with a preponderance of rheumatic heart disease (64% in group I and 68% in group II) as compared with congenital heart disease.

The obstetric outcomes in terms of duration of labor, the timing of delivery, mode of delivery, and rate of cesarean sections with indications is represented in Table II. The mean duration of labor in group I was 7.56±4.806 hours, whereas in group II, it was 7.35±7.440 hours (p=0.906). There was no significant difference observed between the groups in the cesarean delivery rate. The incidence of cesarean section in cardiac pregnancies was 6% in this study. The indications included fetal bradycardia (n=1), meconium-stained liquor (n=1), and deep transverse arrest (n=1). However, none had a cesarean section for a cardiac indication. Nine women (18%) had an instrumental vaginal delivery, either by forceps (16%) or ventouse (2%).

There was no significant difference in the rate of maternal complications observed between the two groups. None of the patients had postpartum complications, and there were no cardiac complications reported such as pulmonary edema or congestive heart failure. There were no maternal deaths in this study. 0

Table 2. Obstetric outcomes

	Group I (n=25)	Group II (n=25)	p value
Duration of labor (hours)			
<12 hours	21 (84%)	21 (84%)	0.999
12-24 hours	4 (16%)	3 (12%)	0.999
>24 hours	0	1 (4%)	0.999
Mean duration of labor (hours)	7.56 (4.806)	7.35 (7.440)	0.906
Delivery during workday hours	13 (52.0%)	6 (24.0%)	0.080
Mode of delivery			
Vaginal delivery	20 (80%)	18 (72%)	0.742
Cesarean section	1 (4%)	2 (8%)	0.999
Instrumental delivery	4 (16%)	5 (20%)	0.999
Indication for cesarean section	1 (4%)	2 (8%)	0.999
Obstetric	0	0	0.999
Cardiac			
Maternal complications			
Obstetric	1	1	0.999
Cardiac	0	0	0.999

(Workday hours were defined as 8.00 am to 5.00 pm)

Table 3. Neonatal outcome

	Group I (n=25)	Group II (n=25)	p value
Birth weight (kg)			
<2.5 kg	6 (24%)	8 (32%)	0.527
2.6-3.0 kg	13 (52%)	13 (52%)	0.999
>3.1 kg	6 (24%)	4 (16%)	0.725
Admission to NICU	0	0	
Stillbirth	0	0	

NICU: Neonatal intensive care unit

The neonatal outcomes in both groups are depicted in Table III. The majority of babies in this study had a birth weight between 2.6-3.0 kg (52%). There was no significant difference in the birth weights of the newborns between the two groups. There were no stillbirths, and none of the babies had an Apgar score <7 at 5 minutes.

Discussion

Minimizing maternal and fetal risks in a pregnant woman with associated heart disease requires the mutual efforts of experienced specialists who are familiar with their management. This team should involve obstetricians, cardiologists, anesthetists, and perinatologists. The timing and mode of delivery, anesthesia and analgesia, cardiac monitoring, and place of delivery should be planned well beforehand. So far, the induction of labor has been performed for obstetric indications only and not the heart disease per se. There is limited literature comparing the outcomes of inductions of labor and spontaneous labor in pregnant women with cardiac disease.

This prospective randomized controlled study was designed to compare maternal and perinatal outcomes between electively induced labor and spontaneous labor in patients with heart disease. This study aimed to determine whether any statistical difference existed between the rate of cesarean delivery, and maternal and neonatal complications in women who did and did not undergo induction of labor. The hypothesis was that the induction of labor was a safe procedure in women with cardiac disease if no significant difference could be demonstrated.

In the present study, 50 consecutive women with heart disease were recruited between 38-41 weeks' gestation after ascertaining eligibility criteria. The decision to induce labor was made jointly by the cardiologist and the obstetric team based on the clinical assessment of each patient's cardiac status and obstetric indications. Induction was performed using oxytocin according to our departmental protocol.

On analyzing the results, it was observed that the majority (34/50) of women were multigravidas. We observed that more patients had rheumatic (66%) rather than congenital heart disease (24%). This is in contrast to recent reports of the west, where congenital heart disease was reported as the primary cause of heart disease in pregnancy. Oron et al.[20] also observed that the principal cardiac lesion in pregnancy was congenital in 72% of patients and in 34 women (28%) it was acquired. However, another Indian study observed rheumatic heart disease as the major contributor (84.6%) as compared with congenital heart disease (15.4%)[21]. The difference can be attributed to geographic factors because congenital heart disease is found to be more common in the west, and acquired, rheumatic heart disease is more prevalent in developing countries such as India.

The present study demonstrated that the mean duration of labor was comparable in both groups. However, 52% of patients delivered during workday hours (between 8:00 am to 5:00 pm) when labor is induced, whereas only 24% of pregnant women delivered during working hours who underwent spontaneous delivery. A similar result has been shown by Oron et al.[20], that 55% of women in the study group delivered during workday hours when the delivery was induced. Therefore, it is concluded that the induction of labor significantly controls the timing of delivery. The timing of delivery matters most in such cases because the team of obstetricians, anesthesiologists, and cardiologists is readily available during workday hours, which is of paramount importance in terms of maternal and perinatal outcome.

In our study, we observed that the majority (94%) had a vaginal delivery, and only one patient underwent cesarean section when labor was induced as compared with two when no induction was performed. Induction of labor does not increase the incidence of cesarean section in pregnant patients with pre-existing heart disease. Oron et al.[20] observed that 63.8% had a vaginal delivery, 21.2% had cesarean section, and 14.8% had instrumental deliveries among 47 patients when labor was induced in cardiac patients, whereas 55.4% had a vaginal delivery, 33.7% had cesarean section, and 10.8% had vaginal deliveries among 74 patients with heart disease who underwent spontaneous labor. The cesarean section rate is, however, less in the present study as compared with the study by Oron et al.[20] The exclusion criteria set for the current study probably contributed to the low cesarean rates. The present study has a similar rate of instrumental deliveries (18%) as observed by Oron et al.[20] (15%). The comparatively higher rate of instrumental deliveries in other studies can be explained by philanthropic efforts to shorten the second stage of labor in order to avoid maternal complications. Pratibha et al.[22], in their study on 200 patients with rheumatic heart disease in pregnancy, concluded that 73.5% had a vaginal delivery and 26.5% had a cesarean section. Thirty women underwent induction of labor for pregnancy-induced hypertension, pregnancy post dates, premature rupture of membranes, and term gestation. Kampman et al.[23], in their study on pregnancy outcomes and deliveries in women with congenital heart disease, observed that the incidence of vaginal delivery was lower in women with congenital heart disease (76.5%) as compared with healthy women (87%), and induction of labor was more common in women with congenital heart disease (37.1%) than in controls (21.4%). Elective cesarean sections were more often performed in women with congenital heart disease (14.1% vs 1.4%), the maximum of which (36.6%) were for cardiac reasons.

Indication for cesarean sections was solely due to obstetric reasons such as fetal bradycardia, meconium-stained liquor, and deep transverse arrest in our study, and none underwent cesarean due to cardiac pathologies, which was consistent with the results of Pratibha et al.[22].

There were no significant maternal complications attributable to induction of labor as well as in patients who underwent spontaneous labor in our study. Only two out of 50 patients had obstetric complications, and none of the study subjects

experienced cardiac complications. In contrast, Pratibha et al.[22] observed cardiac complications in 29 pregnancies (14.5%); 22 (11%) women had cardiac failure. Out of the 22 patients who had cardiac failure, 81.8% (19/22) women were in NYHA III and IV and three women (2.1%) were in NYHA class I and II developed cardiac failure later in pregnancy. However, no maternal mortality was reported in their study. The maternal complication in the form of pulmonary edema and congestive cardiac failure was seen in one case each out of 52 cases by Rangaswamy and Ramachandra[21]. In the present study, the relatively favorable pregnancy outcomes were attributable to the fact that the majority of the patients (96%) were NYHA class I.

In the present study, no statistically significant difference in the rate of neonatal complications between the two groups was observed. The majority of the newborns had a birth weight between 2.6-3.0 kg. There were no stillbirths, and no admissions to the NICU were witnessed in the present study. In contrast, newborn death ensued in 2.8%, and median birth weight was also comparatively lower in offspring of women with congenital heart disease in a study by Kampman et al.[23]. This can be attributed to the small sample size in our study as compared with their study. However, Rangaswamy et al.[21] observed that only 12 out of 52 infants had birth weight <2.5 kg, seven were admitted to the NICU due to meconium aspiration (2 cases), preterm (four cases), and mild birth asphyxia (one case) and there was no perinatal mortality. Pratibha et al.[22] documented 13 perinatal losses (seven IUFD, two stillbirths and four neonatal deaths), the perinatal mortality being 6.4%. The preterm and intrauterine growth retardation rates were 9.3% each. There were 76 low- birth-weight babies (37.43%). The incidence of low birth weight was 34.69% in NYHA class I and II, and 44.64% in class III and IV. Oron et al.[20] found no statistically significant difference in mean birth weight between the patients who underwent induction of labor and those who had spontaneous onset of labor. A total of seven babies needed admission to the NICU. However, no stillbirth was reported.

In the index study, patients were induced with oxytocin instead of prostaglandins. Although oxytocin has to be used guardedly in patients with heart disease, we encountered no adverse effects as observed by Sau et al.[24] who concluded that induction of labor with oxytocin infusion was safe and effective for patients with cardiac disease where elective delivery is warranted. Prostaglandins, however, have the disadvantage that the onset of labor is unpredictable and not clearly related to the state of the cervix. Furthermore, the tablets tend to clump together, and this may result in an uneven absorption of prostaglandin.

Study Limitations

The limited patient numbers and the inclusion of women with only NYHA class I and II were the main limitations of the study. However, a more substantial, randomised, multicenter clinical trial is needed before definitive guidelines can be made for the induction of labor in such group of patients.

Conclusion

This study concluded that induction of labor with oxytocin was a relatively safe procedure in women with low-risk heart disease. The induction does not result in any cardiac complications in pregnant women, and neonatal outcomes is also comparable to those who underwent spontaneous labor. More patients delivered during the daytime when electively induced, which minimized the maternal and fetal risks because the team of obstetricians, anesthesiologists, cardiologists, and perinatologists is readily available during the daytime. Thus, the results of this study may have significant repercussions for the pregnancy management of women with heart disease. Nevertheless, a more substantial, multicenter clinical trial will be indispensable before arriving at any definitive guidelines.

Ethics

Ethics Committee Approval: Clearance from the institute's Ethical Committee was obtained for the study and was performed following ethical standards.

Informed Consent: Was taken.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.D., V.S., Concept: Y.D., V.S., N.A., Design: Y.D., V.S., N.A., Data Collection or Processing: Y.D., V.S., N.A., Analysis or Interpretation: Y.D., V.S., N.A., Literature Search: Y.D., V.S., R.K.D., Writing: Y.D., R.K.D.

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Long bone fractures in neonatal intensive care units of Afyonkarahisar: Five-year's experience

Afyonkarahisar yenidoğan yoğun bakım ünitelerinde tanı konulan uzun kemik kırıkları: Beş yıllık deneyim

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Abstract

Objective: To determine the incidence of long bone fractures and the clinical features related with these fractures diagnosed in neonatal intensive care units (ICUs) within the province of Afyonkarahisar in Turkey.

Materials and Methods: The incidence of clavicular fractures was 2.4 in 1000 live births, and the incidence of femoral fractures was 0.32 in 1000 live births at the neonatal ICUs of Afyonkarahisar.

Results: The incidence of birth trauma-related femoral fracture was 0.16 in 1000 live births, and the incidence of femoral fractures related with osteopenia of prematurity was 1.08 in 1000 live births. The mean gestational age at delivery was 39 weeks, the mean birth weight was 3.308 grams, and the male/female ratio was 3:2 for newborns with birth trauma-related femoral fractures. The mean gestational age at delivery was 30.4 weeks, the mean birth weight was 1256 grams, and the male/female ratio was 2:3 for newborns who had femoral fractures related with osteopenia of prematurity. Breech presentation was present in three newborns (60%), and cesarean section was the type of delivery in all newborns with birth trauma-related femoral fractures.

Conclusion: Cesarean delivery does not reduce the risk for birth trauma-associated femoral fractures, and there is a risk for femoral fracture in cases of emergency cesarean performed for malpresentation. In order to overcome osteopenia of prematurity, calcium, phosphorus, and vitamin D should be supplemented in premature newborns with intrauterine growth retardation and receive long-term total parenteral nutrition.

Keywords: Femoral fracture, intensive care units, metabolic bone diseases, newborn

Öz

Amaç: Bu çalışma, Afyonkarahisar ilinde bulunan üç sağlık merkezindeki yenidoğan yoğun bakım ünitelerinde (YBÜ) tanı konulan uzun kemik kırıklarının görülme sıklığını ve bu kırıklarla ilişkili klinik özellikleri belirlemeyi amaçlamıştır.

Gereç ve Yöntemler: Yenidoğan YBÜ'lerdeki klavikula kırıkları için insidans 2,4/1000 canlı doğum iken femur kırıkları için insidans 0,32/1000 canlı doğum idi.

Bulgular: Doğum travmasına bağlı femur kırıkları için insidans, 1000 canlı doğumda 0,16 iken prematürite osteopenisiyle ilişkili femur kırıkları için insidans, 1000 canlı doğumda 1,08 idi. Doğum travmasına bağlı femur kırığı olguları için ortalama doğum yaşı 39 hafta, ortalama doğum ağırlığı 3308 gram ve erkek/kız oranı, 3:2 olarak bulundu. Prematürite osteopenisiyle ilişkili femur kırığı olguları için ortalama doğum yaşı 30,4 hafta, ortalama doğum ağırlığı 1256 gram ve erkek/kız oranı 2:3 olarak belirlendi. Doğum travmasına bağlı femur kırığı tanısı konulan olguların hepsi sezaryenle doğurtulmuştu ve üç olguda (%60) makat prezentasyonu bulunmaktaydı.

Sonuç: Sezaryenle doğum, doğum travmasına bağlı femur kırığı riskini tamamen ortadan kaldırmadığı gibi malprezentasyon durumunda gerçekleştirilen acil sezaryen durumunda da femur kırığı riski mevcuttur. Uzun süre total parenteral nutrisyon alan ve intrauterin gelişme geriliği tanısı bulunan prematüre bebeklerde, prematürite osteopenisini önlemek için kalsiyum, fosfor ve D vitamini desteği verilmelidir.

Anahtar Kelimeler: Femoral kırıklar, yenidoğan yoğun bakım üniteleri, metabolik kemik hastalıkları, yenidoğan

PRECIS: Neonatal fractures at intensive care.

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Introduction

Bone fractures are rarely encountered in newborns[1]. The incidence of clavicular fractures is about 2 to 3.5 in 1000 live births, whereas the incidence of femoral fracture is 0.13 in 1000 live births[1,2]. Long bone fractures of newborns might occur as a consequence of vaginal delivery-related trauma[2]. Moreover, newborns treated in intensive care units (ICUs) have an increased risk for long bone fractures due to prematurity, low birth weight, and the administration of pharmacologic agents[3]. The incidence of long bone fractures ranges between 1.2% and 10.5% in neonatal ICUs[2,4]. This study aims to specify the incidence of long bone fractures and the clinical characteristics related with these fractures in newborns treated in three ICUs within the province of Afyonkarahisar in Turkey.

Materials and Methods

A total of 54 clavicular fractures and 10 femoral fractures were diagnosed in 31,058 live births at Afyonkarahisar University of Health Sciences Hospital, Afyonkarahisar State Hospital, and Afyonkarahisar Private Park Life Hospital between 2014 and 2019. The incidence of clavicular fracture was 2.4 in 1000 live births, and the incidence of femoral fractures was 0.32 in 1000 live births. Five cases of femoral fractures were related with birth trauma, whereas the remaining five cases were associated with osteopenia of prematurity. Thus, the incidence of birth trauma-related femoral fracture was 0.16 in 1000 live births, and the incidence of femoral fractures related with osteopenia of prematurity was 1.08 in 1000 live births. This is a retrospective review of ten newborns who were diagnosed as having femoral fractures. Data related with perinatal characteristics, treatment procedures, and administered drugs in the ICUs were recorded. Femoral fractures related with birth trauma were defined as fractures that occurred during delivery and were found to be unrelated with postnatal trauma. The remaining femoral fractures were identified as fractures related with osteopenia of prematurity.

Results

Table 1 shows the clinical characteristics of five newborns who were diagnosed as having birth trauma-related femoral fractures and five newborns who had femoral fractures related with osteopenia of prematurity. For the newborns with birth trauma- related femoral fractures, the mean gestational age at delivery was 39 weeks, the mean birth weight was 3308 grams, and the male/female ratio was 3 (60.0%): 2 (40.0%). Breech presentation was present in three newborns (60%) and cesarean section was the type of delivery in all newborns with birth trauma-related femoral fractures. All of these fractures were diagnosed within the first day of life due to the irritability of the newborn and immobility of the involved extremity. For newborns that had femoral fractures related with osteopenia of prematurity, the mean gestational age at delivery was 30.4 weeks, the mean birth weight was 1256 grams, and the male/female ratio was 2 (40.0%): 3 (60.0%). Intrauterine growth retardation (IUGR) was specified in three newborns (60%), bronchopulmonary dysplasia (BPD) was detected in three newborns (60%), and nosocomial sepsis was diagnosed in two newborns (40%) who were diagnosed as having osteopenia of prematurity.

Case #9 was one of the dichorionic twins who had Arnold Chiari type 2 malformation and myelomeningocele. He was diagnosed as having bilateral femoral fractures related with osteopenia of prematurity. The mean duration of hospitalization was 80 days, the mean duration of mechanical ventilation was 38.4 days, and the mean time of diagnosis was 46.4 days for all newborns with femoral fractures related with osteopenia of prematurity. Five newborns with femoral fractures were treated with traction, and three were treated with splinting; the remaining two newborns were treated with both traction and splinting. Complete improvement was noted in 4 weeks for all newborns except one who was lost to follow-up.

Discussion

The incidence of clavicular fractures has been reported as about 2 to 3.5 in 1000 live births, whereas the incidence of femoral fracture has been reported as 0.13 in 1000 live births[1,2]. A Portuguese study detected one or more fractures in 1.1% of neonates who were admitted to the ICU. The most common fracture was clavicle fracture in 60 newborns (79%), followed by skull fracture in 6 newborns (8%) in that study[5]. Similarly, a Welsh study estimated the incidence of fractures as 1.6% for neonatal ICUs. The fracture sites included ribs (n=45), humerus (n=5), ulna (n=3), radius (n=4), femur (n=8), tibia (n=1), clavicle (n=4), and skull (n=1)[1]. As for the present study, the incidence of clavicular fracture was 2.4 in 1000 live births, and the incidence of femoral fractures was 0.32 in 1000 live births. Birth trauma-related bone fractures have been defined as fractures that occur during the first week of life and which are found to be unrelated with postnatal trauma[6]. Malpresentation, preterm delivery, fetal macrosomia, multiple pregnancy, metabolic bone diseases, and emergency cesarean delivery have been identified as the risk factors for birth trauma[7]. Birth trauma-related bone fractures usually appear as a result of the maneuvers performed for breech presentation in vaginal deliveries[6,7]. In the event of malpresentation, cesarean delivery is performed, which has significantly decreased perinatal morbidity and mortality[8]. However, birth trauma occurs in both vaginal and cesarean deliveries, and preferring cesarean section over vaginal delivery does not eliminate the risk of birth trauma[9]. In fact, sudden and careless traction of the newborn's extremities and insufficient myometrial relaxation might cause birth trauma-related bone fractures[8,9]. Hannah et al.[10] reported the incidence of long bone fractures as 0.5% for vaginal deliveries and 0.1% for cesarean deliveries in cases of breech presentation. On the contrary, cesarean deliveries were

associated with a significantly higher incidence of long bone fractures than vaginal deliveries in pregnancies with breech presentation[11-15]. In this study, the incidence of birth trauma-related femoral fracture was 0.16 in 1000 live births. In this study, all newborns with birth trauma-related femur fractures were delivered by cesarean section. The indications for cesarean delivery were malpresentation (breech presentation) in three cases (60%), dystocia in one case (20%), and fetal distress in one case (20%). Two cesarean deliveries performed for dystocia and fetal distress were emergency deliveries. Basha et al.[11] claimed that birth trauma-related bone fractures were diagnosed within a mean time period of 1.5 days. Morris et al.[16] stated that birth trauma-related femoral fractures were diagnosed within a mean time period of 6.3 days. A high index of suspicion may help to make an early diagnosis in newborns that have risk factors for bone fractures because bone fracture-related symptoms may

Table 1. Birth trauma and prematurity related femoral fractures

Case	Gestational age at delivery	Birth weight/ sex	Presentation/ delivery type	Involved extremity	Time of diagnosis	Risk factor	Treatment & duration of improvement
Birth trauma related femoral fractures							
1	38 weeks	3540 grams/ male	Breech/cesarean	Left femur	1st day of life	Meconium stained amniotic fluid	Traction & splinting improvement in 4 weeks
2	39 weeks	2670 grams/ female	Breech/cesarean	Right femur	1st day of life	Emergency cesarean	Traction improvement in 4 weeks
3	39 weeks	3430 grams/ female	Vertex/cesarean	Right femur	1st day of life	Emergency cesarean for dystocia; right patellar subluxation	Traction duration of improvement is unknown
4	38 weeks	3000 grams/ male	Vertex/cesarean	Right femur	1st day of life	Emergency cesarean for fetal distress	Traction improvement in 4 weeks
5	39 weeks	3900 grams/ male	Breech/cesarean	Left femur	1st day of life	-	Traction improvement in 4 weeks
Femoral fractures related with osteopenia of prematurity							
6	29 weeks	770 grams/ female	Vertex/cesarean	Left femur	82nd day of hospital stay (141 days in total)	IUGR, BPD, NEC, nosocomial sepsis, 3 days of steroid use, 121 days of caffeine use, 121 days of TPN	Splinting improvement in 4 weeks
7	28 weeks	1030 grams/ female	Vertex/cesarean	Right femur	67th day of hospital stay (115 days in total)	BPD, PDA, NEC, nosocomial sepsis, 6 days of steroid use, 100 days of diuretic use, 107 days of caffeine use, 86 days of TPN	Traction improvement in 4 weeks
8	28 weeks	1610 grams/ male	Vertex/cesarean	Right femur	66th day of hospital stay (84 days in total)	BPD, PDA, NEC, 5 days of steroid use, 53 days of caffeine use, 60 days of TPN	Splinting improvement in 4 weeks
9	36 weeks	1200 grams/ male	Vertex/cesarean	Right and left femur	6th day of hospital stay (30 days in total)	IUGR	Splinting improvement in 4 weeks
10	31 weeks	1670 grams/ female	Vertex/cesarean	Left femur	11th day of hospital stay (30 days in total)	IUGR, 10 days of caffeine use	Traction & splinting improvement in 4 weeks

IUGR: Intrauterine growth retardation, BPD: Bronchopulmonary dysplasia, NEC: Necrotizing enterocolitis, PDA: Patent ductus arteriosus, TPN: Total parenteral nutrition

be noticed relatively late[11,16]. In this study, all newborns with birth trauma-related femur fractures were diagnosed within the first day of life. Osteopenia of prematurity is also known as a metabolic bone disease of prematurity. This clinical entity is specified when postnatal bone mineralization is significantly lower than intrauterine bone mineralization adjusted for gestational age. The incidence for osteopenia of prematurity increases as gestational age and birth weight decrease[17]. Wei et al.[1] reported that the neonates who had non-traumatic fractures had significantly lower gestational age and birth weight. Osteopenia, need for multiple medical interventions, and late diagnosis of fractures were significantly more frequent in neonates who had non-traumatic fractures. Osteopenia of prematurity usually appears between the 6th to 12th weeks of corrected gestational age. This clinical entity affects 20% to 30% of newborns weighing less than 1500 grams and up to 50% to 60% of newborns weighing less than 1000 grams[5,17]. Although its exact incidence is unknown, bone fractures related with osteopenia of prematurity appear in 1.2% to 10% of very-low-birthweight newborns[18,19]. In this study, the incidence of osteopenic femoral fractures was 1.08 in 1000 live births.

Preterm delivery; IUGR; necrotizing enterocolitis; insufficient intake of calcium, phosphorus and vitamin D; and long-term administration of total parenteral nutrition (TPN) and calcium and phosphorus losing drugs (diuretics, steroids and caffeine) have all been addressed as the risk factors for osteopenia of prematurity[20]. Preterm delivery interrupts this accumulation and induces osteopenia of prematurity because the majority of fetal calcium and phosphorus accumulation takes place in the last trimester[20]. All of the newborns with femur fractures related with osteopenia of prematurity were born before the 37th gestational week. The administration of TPN over two weeks has been identified as a risk factor for osteopenia in premature newborns with very low birth weight[21]. Three out of five newborns who had femur fractures related with osteopenia of prematurity had received TPN treatment (60%). Caffeine use triggers osteopenia of prematurity by inducing demineralization and calciuria[18,20]. Four out of five newborns who had femur fractures related with osteopenia of prematurity had a history of caffeine intake (80%). BPD also leads to osteopenia of prematurity by inducing demineralization[22]. BPD was detected in three out of five newborns with femur fractures related with osteopenia of prematurity (60%). The existence of IUGR has been denoted as a factor that independently increases the risk for osteopenia of prematurity by ten times[23]. This finding can be attributed to the significantly lower maternal and fetal calcium intake, significantly higher serum parathormone levels, and significantly lower vitamin D levels in umbilical cord blood[24-27]. Accordingly, IUGR was determined in three out of five newborns who had femur fractures related with osteopenia of prematurity (60%).

Osteopenia of prematurity usually presents with non-specific clinical symptoms. There are no specific criteria for diagnosing osteopenia of prematurity. Several biochemical markers have frequently been used as screening tools and diagnostic markers, but threshold values vary widely. Standard X-ray is generally used to diagnose osteopenia of prematurity, but this method cannot detect osteopenia unless bone mineralization is reduced by 20%[28]. Femoral fractures of newborns can be treated with either traction or splinting[15]. In this study, five newborns with femoral fractures were treated with traction, three newborns were treated with splinting, and two newborns had a combination of splinting and traction. When treated, neonatal femur fractures have an excellent prognosis[15]. Thus, all newborns in this study improved without any sequelae.

Study Limitation

The power of the present study is limited by its retrospective design, relatively small cohort size, and the lack of data related to the long bone fractures diagnosed in newborns who were admitted to outpatient clinics.

Conclusion

Malpresentation, preterm delivery, fetal macrosomia, multiple pregnancy, metabolic bone diseases, and emergency cesarean delivery have been defined as the risk factors for birth trauma-related bone fractures. Birth trauma occurs in both vaginal and cesarean deliveries and preferring cesarean section over vaginal delivery does not eliminate the risk of birth trauma. Preterm delivery, IUGR, necrotizing enterocolitis, insufficient intake of calcium, phosphorus and vitamin D, long-term administration of TPN and calcium and phosphorus losing drugs (diuretics, steroids and caffeine) have all been addressed as the risk factors for osteopenia of prematurity. In order to overcome osteopenia of prematurity, calcium, phosphorus, and vitamin D should be supplemented in premature newborns that have IUGR and receive long-term TPN.

Ethics

Ethics Committee Approval: This study was approved by the Research Ethics Committee of the Afyonkarahisar University of Health Sciences Faculty of Medicine (Grant no: 2019/52-9).
Informed Consent: Was taken.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K.P., A.A.K., Concept: A.A.K., Design: A.A.K., Data Collection or Processing: H.K., Analysis or Interpretation: M.K.P., Literature Search: H.K., Writing: H.K., M.K.P.
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The prevalence of uterine anomalies in infertile patients with polycystic ovary syndrome: A retrospective study in a tertiary center in Southeastern Turkey

İnfertil polikistik over sendromu hastalarında uterus anomalilerinin prevalansı: Türkiye'nin Güneydoğusunda bulunan üçüncü basamak bir merkezde retrospektif çalışma

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Abstract

Objective: To evaluate the prevalence of uterine anomalies in infertile patients with polycystic ovary syndrome (PCOS) admitted to our tertiary hospital in Southeastern Turkey.

Materials and Methods: The files of 3033 patients with infertility who presented to the infertility polyclinics were retrospectively analyzed, and uterine anomalies were detected in 131 patients. Seven hundred ten of these patients were evaluated as having PCOS, 55 of whom had uterine anomalies. Patients with PCOS were also divided into two subgroups as those with primary and secondary infertility.

Results: Of the 3033 patients with infertility who were evaluated, 57 (8%) of 710 infertile patients with PCOS, and 74 (3%) of 2323 non-PCOS patients with infertility had uterine anomalies. A statistically significant difference was found between the two groups ($p<0.001$), and no significant difference was found between the primary and secondary infertile PCOS subgroups ($p=0.3$). Septate uteri and arcuate uteri had a high prevalence in the PCOS group, and no t-shaped or hypoplastic uteruses were observed in this group.

Conclusion: To or knowledge, this is the first study in our region to examine the relation between PCOS and Müllerian anomalies. We demonstrated uterine anomalies and their prevalence in patients with infertility. A more careful examination is required in order to determine the incidence of uterine anomalies in patients with PCOS.

Keywords: Uterine anomaly, polycystic ovary syndrome, infertility

Öz

Amaç: Bu çalışmada, polikliniğimize başvuran infertil polikistik over sendromu (PKOS) hastalarında, uterus anomalilerinin prevalansı değerlendirildi.

Gereç ve Yöntemler: Şubat 2017 ve Mayıs 2019 tarihleri arasında Gazi Yaşargil Eğitim ve Araştırma Hastanesi İnfertilite polikliniğine başvuran 3.033 infertil hastanın dosyaları geriye dönük olarak incelendi ve 131 hastada uterus anomalisi saptandı. Yedi yüz on hasta PKOS olarak değerlendirildi ve 57'sinde uterus anomalisi vardı. Ayrıca PKOS hastaları primer ve sekonder olarak 2 alt gruba ayrıldı.

Bulgular: Üç bin otuz üç infertil hasta değerlendirildi. Yedi yüz on infertil PKOS hastanın 57'sinde (%8) ve PKOS olmayan 2,323 infertil hastanın 74'ünde (%3) uterus anomalisi saptandı. İki grup arasında istatistiksel olarak anlamlı bir fark bulundu ($p<0,001$). Primer ve sekonder infertil PKOS alt grupları arasında anlamlı bir fark bulunmadı ($p=0,3$). Septus uteri ve arkuat uteri, PKOS grubunda yüksek prevalansa sahipken, bu grupta t-shaped ve hipoplastik uteri gözlenmedi.

Sonuç: İnfertil hastalarda temsil edilen uterin anomalilerin farklı kategorilerini gösterdik. PKOS nedeniyle kliniğimize başvuran hastalar anomali açısından dikkatli incelenmelidir. Müdahale gerektiren durumların ve infertilite ile ilgili sorunların daha önce çözülmesini sağlayan bir farkındalık yaratacaktır.

Anahtar Kelimeler: Uterin anomali, polikistik over sendromu, infertilite

PRECIS: This is the first study in our region that we know of that offers an examination of the relation between polycystic ovary syndrome and Müllerian anomalies.

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous, multifactorial disease affecting 10% of the female population of reproductive age. Hyperandrogenism, ovulatory dysfunction, and polycystic ovary images are among the main features of PCOS[1,2]. Congenital uterine anomalies occur as a result of a defect in the Müllerian canals. Genetic, sporadic or multifactorial factors are thought to play a role in the formation of Müllerian duct anomalies. Uterine anomalies cause decrease pregnancy rates and increase the risk of miscarriage and preterm birth[3-5]. The high rates of PCOS and uterine anomalies in patients with infertility suggest that there may be a relationship between them. This study aimed to evaluate the prevalence of uterine anomalies in infertile patients with PCOS in a tertiary center in Southeast Turkey.

Materials and Methods

The files of 3033 infertile patients who presented to the infertility polyclinics of Gazi Yaşargil Training and Research Hospital in the Southeast part of Turkey between February 2017 and May 2019 were retrospectively analyzed, and uterine anomalies were detected in 131 patients. Of all the infertile patients, 710 were evaluated as having PCOS, and 57 had uterine anomalies. Patients with PCOS were divided into two subgroups, as those with primary or secondary infertility.

The patients were evaluated according to the Rotterdam criteria: 1: ultrasound examination; 2: clinical and biochemical evidence of hyperandrogenism; and 3: oligoovulation/anovulation[6].

All patients with myoma, ovary cysts, tubular blockage, and male-factor infertility were excluded. The patients were first evaluated using transvaginal ultrasonography. Standard steps hysterosalpingography, laparoscopy, hysteroscopy, and magnetic resonance imaging were performed to confirm the diagnosis of uterine anomalies. The American Fertility Society classification was used to diagnose uterine anomalies[7]. The study was approved by the Local Ethics Committee of University of Health Sciences Gazi Yaşargil Training and Research Hospital (approval number: 318).

Statistical Analysis

The data of nominal variables are summarized in the form of frequency or percentages. Comparative data were compared using the chi-square test. Any differences were considered significant for p values smaller than 0.05. All statistical analyses were performed using R-software v.3.5.1 (R statistics software, Institute for Statistics and Mathematics, Vienna, Austria).

Results

Of the 3033 patients with infertility who were evaluated, 57 (8%) of the 710 infertile patients with PCOS and 74 (3%) of the 2323 non-PCOS patients with infertility had uterine anomalies. Septate uteri and arcuate uteri had a high prevalence in the PCOS group, and no t-shaped or hypoplastic uteruses were observed in this group (Table 1). A statistically significant difference was found between the two groups (p<0.001), and no significant difference was found between the primary and secondary infertile PCOS subgroups (p=0.3) (Table 2).

Table 1. Distribution of uterine anomalies

Type of uterine anomaly	PCOS	Non-PCOS	Total	p value
	(n=710)	(n=2323)	(n=3033)	
Bicornuate	3-0.4%	7-0.3%		0.6
Didelphys	2-0.3%	1-0.04%		0.07
Septate	19-3%	13-0.5%		<0.001
Arcuate	30-4%	45-2%		0.003
T-shaped	0-0	2-0.08%		0.4
Unicornuate	3-0.4%	4-0.2%		0.2
Hypoplasis	0-0	2-0.08%		0.4
Total anomalies	57-8%	74-3%	131	<0.001

PCOS: Polycystic ovary syndrome

Table 2. Distribution of cases with polycystic ovary syndrome and uterine anomalies

PCOS	Primary infertile	Secondary infertile	Total	p value
	(n=523)	(n=187)	(n=710)	
Uterine anomalies	45-9%	12-6%	57	0.3

PCOS: Polycystic ovary syndrome

Discussion

It is necessary to diagnose uterine anomalies because of their different structural features[8]. The incidence of uterine anomalies was reported as 6.7% in fertile patients, 7.3% in infertile patients, and 16.7% in recurrent abortions[9]. In the present study, the prevalence of uterine anomalies in infertile patients with PCOS admitted to our hospital was evaluated retrospectively, and a significant relationship was observed between PCOS and uterine anomalies. The reproductive system, except the ovaries, consists of müllerian channels. The uterus, which is composed of mullerian canals, is initially separated by a septum, then fusion occurs when the intervening septum disappears[5]. It is thought that the uterine septum is regressed by mediation of the *Bcl-2* gene[10]. Defects in the formation, convergence or regression of the Müllerian ducts can cause different anomalies. Several studies have shown significantly higher anti-Müllerian hormone (AMH) levels in patients with PCOS. This could be linked to coexisting uterine anomalies because AMH definitely plays a role during early life in the degeneration of Müllerian ducts[11-13]. In the present study, a significant relationship was noted between PCOS and

uterine anomalies. Developmental defects could be a possible cause for both PCOS and uterine anomalies. Hormonal changes such as AMH may play a role in the etiopathogenesis of both conditions. MacDougall and Ultrasonographer[14] noted that in 1512 women with PCOS, two cases of Müllerian anomalies were reported, and their prevalence in patients with PCOS and the general population was not different. Similarly, Acién[15] found no such relationship of polycystic ovarian disease in women with uterine malformations. In contrast, Appelman et al.[16] found a significant relationship between PCOS and uterine Müllerian anomalies. In a retrospective study, it was reported by Ugur et al.[17] that PCOS and Müllerian anomalies occurred as a result of a common. Finally, Saleh and Shawky Moiety[18] found a relationship between PCOS and uterine anomalies in a prospective study of patients with infertility. In addition, uterine anomaly classification, arcuate uteri was the most common anomaly in PCOS patients, followed by septum uteri. Similarly, in this study, septate uteri and arcuate uteri had a high prevalence level in the PCOS group. Christiansen and Detti[19] showed that surgical correction was warranted for Müllerian anomalies that caused pregnancy loss, such as septate and subseptate (i.e., arcuate) uteri. The minimum subseptation length that indicates a surgical incision is still debated; however, authors advocated a 5.9 mm cut-off and proposed that it be adopted, especially when a history of pregnancy loss is present or when fertility treatments are planned.

The limitation of the study is that the prevalence of PCOS did not reflect the general population because the patient sample mainly comprised women with infertility. This may be due to the fact that patients without PCOS with Müllerian anomalies do not present as infertile. It should also be noted that it is not easy to perform an epidemiologic study on the prevalence of Müllerian anomalies because they are not common in the general population, and most go undiagnosed because they are asymptomatic. To our knowledge, this is the first study in our region to examine the relation between PCOS and Müllerian anomalies. We demonstrated uterine anomalies and their prevalence in patients with infertility. A more careful examination is required in order to determine the incidence of uterine anomalies in patients with PCOS.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of University of Health Sciences Gazi Yaşargil Training and Research Hospital (approval number: 318).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Platelet-rich plasma administration to the lower anterior vaginal wall to improve female sexuality satisfaction

Kadın cinselliğinde memnuniyeti artırmak için alt ön vajinal duvara trombositçe zengin plazma uygulaması

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Abstract

Objective: To investigate the effect of platelet-rich plasma (PRP) injection to the lower one-third of the anterior vaginal wall on sexual function, orgasm, and genital perception in women with sexual dysfunction.

Materials and Methods: Four sessions of PRP were administered to the anterior vaginal wall of 52 female patients with sexual dysfunction and orgasmic disorder [Female Sexual Function Index (FSFI) total score ≤26 orgasmic subdomain score ≤3.75]. Prior to the PRP administrations in each session, the FSFI validated in Turkish, the Female Genital Self-Image Scale (FGSIS), the Female Sexual Distress Scale-Revised (FSDS-R), and Rosenberg's Self-Esteem Scale were used and in the final follow-up, and the Patient Global Impression of Improvement (PGI-I) was performed and the results were analyzed.

Results: Following the application of the PRP, the total FSFI score was observed as 27.88±4.80 and the total score was 26 and above in 50% of the patients (p<0.001). Orgasm subdomain scores were found as 2.11±1.20 before the PRP treatment and 4.48±1.14 afterwards (p<0.001). A significant change was observed in all sub-domains after PRP and it was observed that this change started after the first administration (p<0.001). A statistically significant increase was determined in FGSIS genital perception scores, which was significant between the 1st and 2nd months (p<0.001). The FSDS-R scores showed a minimal increase in stress scores as the application number increased, but a statistically significant decrease was observed in the 4th administration (p<0.001). No statistically significant difference was found in Rosenberg Scale scores before and after treatment (p=0.389). High satisfaction was found in PGI-I scores.

Conclusion: As a minimally invasive method, PRP administration to the distal anterior vaginal wall may improve female sexuality with high satisfaction.

Keywords: Platelet-rich plasma, female sexual dysfunction, female orgasmic disorder

Öz

Amaç: Bu çalışmanın amacı, seksüel disfonksiyonu olan kadınlarda vagen alt 1/3 anterior duvarına trombositten zengin plazma (PRP) enjeksiyonunun seksüel fonksiyonlara etkisini araştırmaktır.

Gereç ve Yöntemler: Seksüel disfonksiyon ve orgazmik bozukluk olan [Female Sexual Function Index (FSFI) total skor 26 ve altı, orgazmik subdomain skor 3,75 ve altı] 52 kadın hastanın vagen anterior duvarına 4 seans PRP uygulandı. PRP öncesi ve her seansta Türkçe'ye valide edilmiş FSFI, the Female Genital Self-Image Scale (FGSIS), The Female Sexual Distress Scale-Revised (FSDS-R), Rosenberg's Self-Esteem Scale uygulandı ve son kontrolde Patient Global Impression of Improvement (PGI-I) uygulandı, sonuçlar analiz edildi.

Bulgular: PRP sonrası, FSFI total skor 27,88±4,80 [anlamlılık ± standart sapma (SD)], %50'de total skorun 26 ve üzerinde olduğu görüldü (p<0,001). Orgazm subdomain skorları PRP öncesi 2,11±1,20 (anlamlılık ± SD), sonrası 4,48±1,14 (anlamlılık ± SD) saptandı (p<0,001). PRP sonrası tüm alt domainlerde anlamlı değişim saptandı ve bu değişimin 1. uygulamadan sonra başladığı görüldü (p<0,001). FGSIS genital algı skorlarında da istatistiksel olarak anlamlı artış saptandı, bu artışın 1.-2. ay arasında belirgin olduğu görüldü (p<0,001). FSDS-R skorlarında uygulama arttıkça stres skorunda minimal artış görüldü ancak 4. uygulamada istatistiksel anlamlı düşüş saptandı (p<0,001). Rosenberg Skala'da tedavi öncesi ve sonrası istatistiksel olarak anlamlı fark saptanmadı (p=0,389). PGI-I skorlarında hastalarda yüksek memnuniyet saptandı.

Sonuç: Minimal invaziv bir metot olarak, distal anterior vajinal duvarına PRP uygulaması, kadın cinselliğinde yüksek memnuniyet sağlayabilir.

Anahtar Kelimeler: Trombositten zengin plazma, kadın cinsel işlev bozukluğu, kadın orgazm bozukluğu

PRECIS: PRP administration to the lower anterior vaginal wall to improve female sexuality.

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Introduction

Platelet-rich plasma (PRP) treatment aims to increase the self-healing ability of the human body by increasing neovascularization and collagen formation through the effect of high concentration autologous growth factors administered to the tissue[1]. The most important advantages are it being autologous and reliable[2,3]. Case series, pilot studies, and case reports related to gynecologic use of PRP are observed in the literature[2,3]. PRP has been used in atrophic diseases such as lichen sclerosis in the vagina, stress urinary incontinence, episiotomy scars, and lubrication disorders in the vagina[2]. The first use for sexual dysfunction in women was performed by Charles Runels under the name of O-Shot[3]. Improvement of sexual functions was reported with PRP administration to the G-spot. Sexual dysfunction classification systems are structured on the coordination of the phases including the desire, arousal and orgasm of the sexual response cycle, which is defined by Master and Johnson and developed by Kaplan[4]. According to the criteria of the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-5), desire/arousal and orgasmic disorders are the most common causes of female sexual dysfunctions[5]. Sexual stimulation of women is known to be provided mostly by touching[6]. The most sensitive point to stimulus in the female body is the genital area[6]. In the vagina, the lower one-third of the anterior region has been proved to have more nerves immunohistochemically[7-9] and it is known that the response of the distal anterior vaginal wall to contact and to pressure is higher than the other part of the vagina, in penis-vagina penetration[6,10]. PRP treatment has the potential to be part of a surgical and non-hormonal approach in patients with sexual dysfunction with regenerative changes by increasing collagen formation and neovascularization in the anterior vaginal wall. The aim of this study was to investigate the effect of PRP injections to the lower anterior vaginal wall on sexual function, orgasm, and genital perception of patients with sexual dysfunction.

Materials and Methods

Our retrospective cross-sectional study was performed on 52 patients who fulfilled the criteria for inclusion and exclusion and who were admitted to a private clinic due because of sexual dysfunction between December 2017 and April 2019 and administered vaginal PRP.

The inclusion criteria of our study included

Patients aged over 18 years of age, who had been sexually active for the last 12 months and had had sexual intercourse at least every 15 days, dyspareunia, who could not achieve orgasm, had difficulty in orgasm, had dryness and pain in intercourse in the last six months, absence of sexual appetite, those who met the diagnostic criteria of female sexual interest/arousal disorder or orgasmic disorder according to the DSM-5, and patients with a total of 26.5 and six points and whose orgasm subdomain is below 3.75 in the Female Sexual Function Index (FSFI) questionnaire completed in the first admission, were included in the study. Patients who were sexually inactive, who had organic pathology in vaginal examination, who were pregnant, aged under 18 or over 55 years, postmenopausal, using antidepressant-psychotropic drugs, using sexual strengthening medication such as sildenafil, using topical estrogen in the past year, using the contraceptive pill, alcohol and drug addicts, those who had undergone vaginal aesthetic and genital oncological surgery, receiving chemotherapy or radiotherapy, those whose FSFI questionnaire scores were above 26.5, and those with orgasm subdomains above 3.75 were excluded from the study. Primary inorgasmic patients were excluded from the study. The patients were asked whether their spouses had erectile dysfunction and premature ejaculation; respondents who said "yes" were excluded from the study. The Beck Depression Inventory was applied to the patients who met the inclusion criteriai and patients with a score of 17 and above were excluded from the study.

Scales

The following questionnaires were administered before the PRP administration in the 1st, 2nd, 3rd, and 4th month and at the 6th month follow-up. The validated Turkish version of the FSFI, Female Genital Self-Image Scale (FGSIS), Female Sexual Distress Scale-Revised (FSDS-R), and the Rosenberg Self-Esteem Scale (RSES) were applied to all participants[11-14]. The Patient Global Impression of Improvement (PGI-I) was completed at the 6th month for the evaluation of the patient's satisfaction. The FSFI is a brief instrument for the assessment of sexual function that consists of 19 questions and has been validated based on DSM-4 diagnoses of desire disorder, arousal disorder, and orgasmic dysfunction. Questions are scored for domains of libido, arousal, lubrication, orgasm, satisfaction, and pain[15]. The cut-off scores for the FSFI scales were created by using the scale-specific means for women without sexual dysfunctions minus one standard deviation (SD) as reported by Wiegel et al[16]. The cut-off scores were as follows: 3.16 for desire, 3.97 for arousal, 4.31 for lubrication, 3.75 for orgasm, 3.85 for sexual satisfaction, and 4.22 for pain. An FSFI total score of 26.55, out of a maximum possible score of 36, is generally considered to be the optimal cut-off score to differentiate women with and without sexual dysfunction[16]. The seven-item FGSIS assesses women's feelings and beliefs about their own genitals and has established reliability and validity in a convenience sample[17]. Respondents' scores on each item were summed for a total sum score ranging from 7 to 28, with higher scores indicating a more positive genital self-image. The FSDS-R assesses different aspects of sexual-activity-related distress in women. The total score, ranging from 0 to 52, can be computed by adding all 13 item scores. Higher scores indicate higher levels of sexual distress[13]. The RSES, developed by Rosenberg, is one of the most widely used tools to assess global self-esteem by measuring

both positive and negative individual feelings[18]. The RSES is a 10-item, 4-point Likert-type Scale, ranging between 0 and 30. Scores between 15 and 25 are within the normal range, and scores above 25 are indicative of high self-esteem. PGI-I is a global index that may be used to rate the response of a condition to therapy. It is a simple, direct and easy-to-use scale that is intuitively understandable to both physicians and patients, and has a single question for comparing now and before beginning the application on a scale from 1: very much better, to 7: very much worse.

Application

The patients were informed on how the PRP application would be performed, together with the possible risks and complications. The procedure was performed after written informed consent was obtained.

The patients were placed on the operating table in the dorsal lithotomy position when the bladder was empty, half an hour before the procedure for applying a local anesthesia cream which contained lidocaine 2.5% and prilocaine 2.5%. The local anesthetic was administered around the clitoris and the vaginal lower one-third of the region.

A PRP kit was used for the PRP administration. The PRP kit consists of 2 PRP tubes, re-suspension tube, 2 injectors, and 2 needles in a single sterile mold. Each PRP tube has a volume of 10 mL and contains 1 mL of citrate.

The PRP+public-private partnership (PPP) preparation steps in our study were as follows:

i. Approximately 18 mL of venous blood from the cubital region of the upper extremity of the patients were taken to two special vacuumed CE-certified PRP tubes;
ii. The collected blood was centrifuged at 3200 rpm for eight minutes;
iii. After centrifugation, it was found that the plasma (upper layer), platelets and leukocytes (middle layer called "Buffy coat") and erythrocytes (lowest layer) were divided into three layers;
iv. Approximately 2 mL of PRP and 2-3 mL PPP from each tube was transferred to the re-suspension tube;
v. For a more homogeneous spread of platelets in the re-suspension tube, the tube was shaken gently by hand for 30 sec;
vi. After isolation of the 4-5 mL of PRP+PPP from the re-suspension tube, calcium chloride (0.5 mL) was added, thereby activating the thrombin cascade, causing degranulation of platelets, releasing growth factors and cytokines, and starting the transformation to the platelet-rich fibrin matrix;
vii. A total of 9 mL PRP+PPP mixture was obtained from both tubes;
vii. After the CaCl was added, the treatment was performed within 5 min;
ix. The PRP+PPP was now ready for injection;
x. PRP+PPP injected using a 21 gauge (G) needle.

Patients were placed the lithotomy position again and PRP was administered to form pili of 4 cc around the clitoris in the direction of clock positions of 12, 3, 6, and 9, each with 1 cc, 2 cc subcutaneously; right/left of paraurethral vaginal wall each with 1 cc, 3 cc; and mid-urethral midline/right/left 1 cc. The PRP applications were administered using 31-G needles (Figure 1).

This administration was continued once every four weeks, for four months. The patients were evaluated by repeating the questionnaires in each administration and at the 6th month.

Statistical Analysis

The descriptive statistics for continuous variables are expressed as mean ± standard deviation. Continuous data were analyzed using repeated measures analysis of variance followed by Bonferronni post-hoc tests. A p value <0.01 was considered statistically significant. Statistical analysis was performed using the SPSS for Windows version 22 software (SPSS Inc., Chicago, IL, USA). The institutional Ethics Committee approved the study, and written informed consent was obtained from all individual participants included in the study.

Results

The mean age of the patients was 37.5±9.8 (mean ± SD; 22-55 minimum-maximum) years. The mean body mass index was 26.54±5.10 (mean ± SD; 18.29-40.00 minimum-maximum) kg/m^2, 100% wer sexually active, 21% were single, and 79% were married. The results of the PRP before, during, and after the administration for 4 months are seen in Table 1 and Figures 2-5. The pre-treatment FSFI total score was 13.61±3.78 (mean ± SD), and the total score of all patients was below 26. The subdomains of FSFI of desire, arousal, lubrication, orgasm, satisfaction and pain mean scores (mean ± SD) were found as 3.29±1.35, 2.35±1.37, 2.25±1.37, 2.11±1.20, 1.92±1.52, and

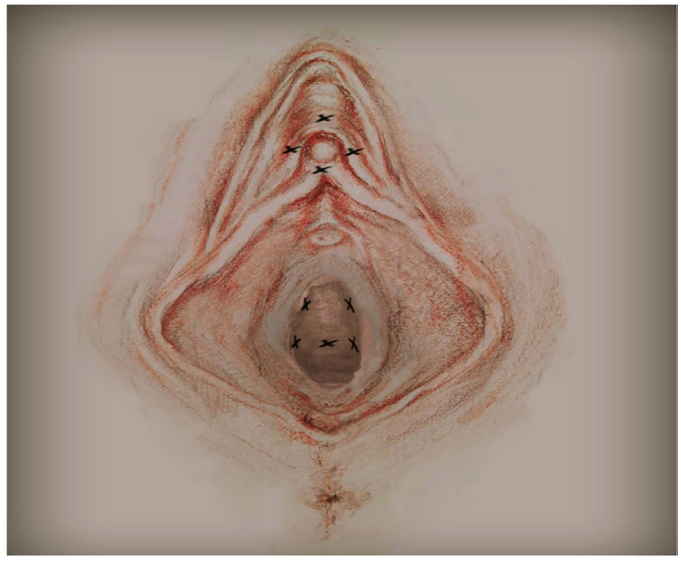

Figure 1. The application areas to the lower anterior vaginal wall and around the clitoris.

1.66±1.18, respectively. The pre-treatment FGSIS scores were 17.44±5.58 (mean ± SD), FSDS-R scores were 19.17±12.00 (mean ± SD), and RSES scores were found as 20.96±6.08 (mean ± SD) (Table 1).

After application of the PRP, the total score of FSFI was 27.88±4.80 (mean ± SD) and the total score was 26 and over in 50% of cases. The increase in the total score was found statistically significant after the 1st month (p<0.001, Figure 2). After PRP, the subdomains of FSFI of desire, arousal, lubrication, orgasm, satisfaction, and pain mean scores were found as (mean ± SD); 4.99±0.83, 4.66±1.03, 4.52±0.94, 4.48±1.14, 4.71±0.87, and 4.50±1.06, respectively. A significant initial change in all subdomains was observed after the first administration (p<0.001, Figure 3). The FGSIS scores after treatment were 23.90±2.12 (mean ± SD), FSDS-R scores were 11.38±1.91 (mean ± SD), and RSES scores were 22.05±5.70 (mean ± SD) (Table 1, Figures 2-5). No statistically significant difference was observed in the Rosenberg scale before and after treatment (p=0.389).

A statistically significant increase was determined in FGSIS genital perception scores, which was prominent between the 1st and 2nd months (p<0.001, Figure 4). FSDS-R scores showed a minimal increase in the stress score as the application number increased, but a statistically significant decrease was observed between the 1st and 4th months (p<0.001, Figure 5). As a result, it was seen that there was a significant improvement in scale scores after four PRP administrations, except on the RSES.

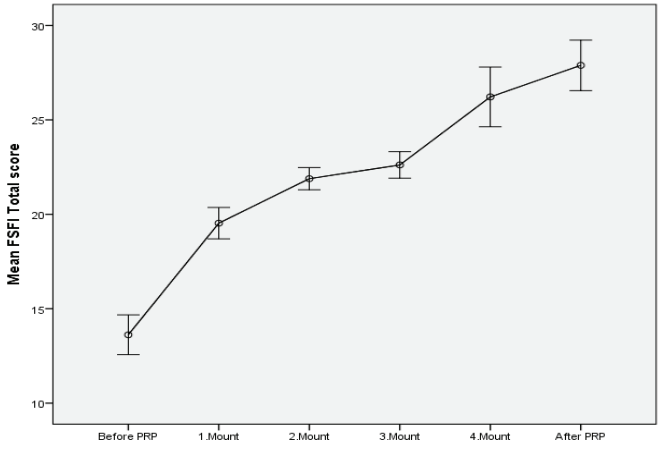

Figure 2. Graphs of Mean Female Sexual Function Index

Total score at application times. Bars are standard deviation. After PRP; 6th month checks are shown.

FSFI: Female Sexual Function index, PRP: Platelet-rich plasma

Discussion

In this study, we aimed to investigate the effect of PRP injection to the lower anterior vaginal wall on sexual function, orgasm, and genital perception of patients with sexual dysfunction. With significant changes in the results of the survey, we found that a positive effect started in the first session of PRP. We found an increase in the satisfaction of the patients after the procedure. PRP is based on the separation of a small amount of blood taken from the patient into a special tube, after which centrifugation is performed and the obtained "PRP" is returned to the same patient by injection. PRP has been used in many areas such as cosmetic use, wound healing, and urologic and orthopedic applications[1-3]. In many medical diseases, it is becoming increasingly popular among minimally invasive methods with a wide area of use. It is known that PRP increases collagen formation and neovascularization with growth factors (platelet-derived growth factor, transforming growth factor-β, epidermal growth factor) by 5-10 times more than normal blood. PRP therapies aim to increase the self-healing ability of the human body due to the effects of high-concentration autologous growth factors applied to the tissue[1]. In the literature, it is observed that PRP use in gynecology is less than in other disciplines,

Table 1. Analysis of the questionnaires, stratified by duration: before platelet-rich plasma, during and after platelet-rich plasma

n=52	Before PRP mean ± SD	First application mean ± SD	Second application mean ± SD	Third application mean ± SD	Fourth application mean ± SD	After PRP mean ± SD	p
Rosenberg Scale	20.96±6.08	21.15±4.61	20.94±5.50	22.01±4.32	22.76±5.37	22.05±5.70	0.389
FSDS-R	19.17±12.00[a]	15.63±9.32[a]	16.34±10.72[a]	18.19±8.84[a]	20.25±7.33[a]	11.38±1.91[b]	<0.001
FGSIS	17.44±5.58[a]	17.82±4.88[a]	21.42±4.96[b]	21.94±3.32[c]	24.21±2.48[d]	23.90±2.12[d]	<0.001
FSFI							
Total scores	13.61±3.78[a]	19.53±2.98[b]	21.88±2.12[c]	22.61±2.51[c]	26.21±5.67[d]	27.88±4.80[d]	<0.001
Desire	3.29±1.35[a]	3.97±1.07[b]	4.30±0.80[b]	4.38±0.76[b]	4.69±1.08[b]	4.99±0.83[c]	<0.001
Arousal	2.35±1.37[a]	3.21±1.13[b]	3.48±1.11[b]	3.67±0.94[b]	4.44±1.21[c]	4.66±1.03[c]	<0.001
Lubrication	2.25±1.37[a]	3.23±2.92[a,b]	3.33±0.91[b]	3.49±1.00[b]	4.30±1.08[c]	4.52±0.94[c]	<0.001
Orgasm	2.11±1.20[a]	2.99±1.08[b]	3.48±1.00[b,c]	3.66±1.01[c,d]	4.28±1.31[d]	4.48±1.14[d]	<0.001
Satisfaction	1.92±1.52[a]	3.58±1.11[b]	4.07±0.77[b,c]	4.19±0.90[c,d]	4.46±1.05[c,d]	4.71±0.87[d]	<0.001
Pain	1.66±1.18[a]	2.90±0.78[b]	3.13±0.84[b]	3.15±0.85[b]	3.99±1.34[c]	4.50±1.06[c]	<0.001

Each subscript letter denotes a subset of categories whose column proportions do not differ significantly from each other at the 0.05 level. After PRP; 6th month checks are shown.
PRP: Platelet-rich plasma, SD: Standard deviation, FSFI: The Female Sexual Function index (2-36), FSDS-R: The Female Sexual Distress Scale-Revised (0-52), Rosenberg's Self-Esteem Scale (0-30), FGSIS: The Female Genital Self-Image Scale (7-28)

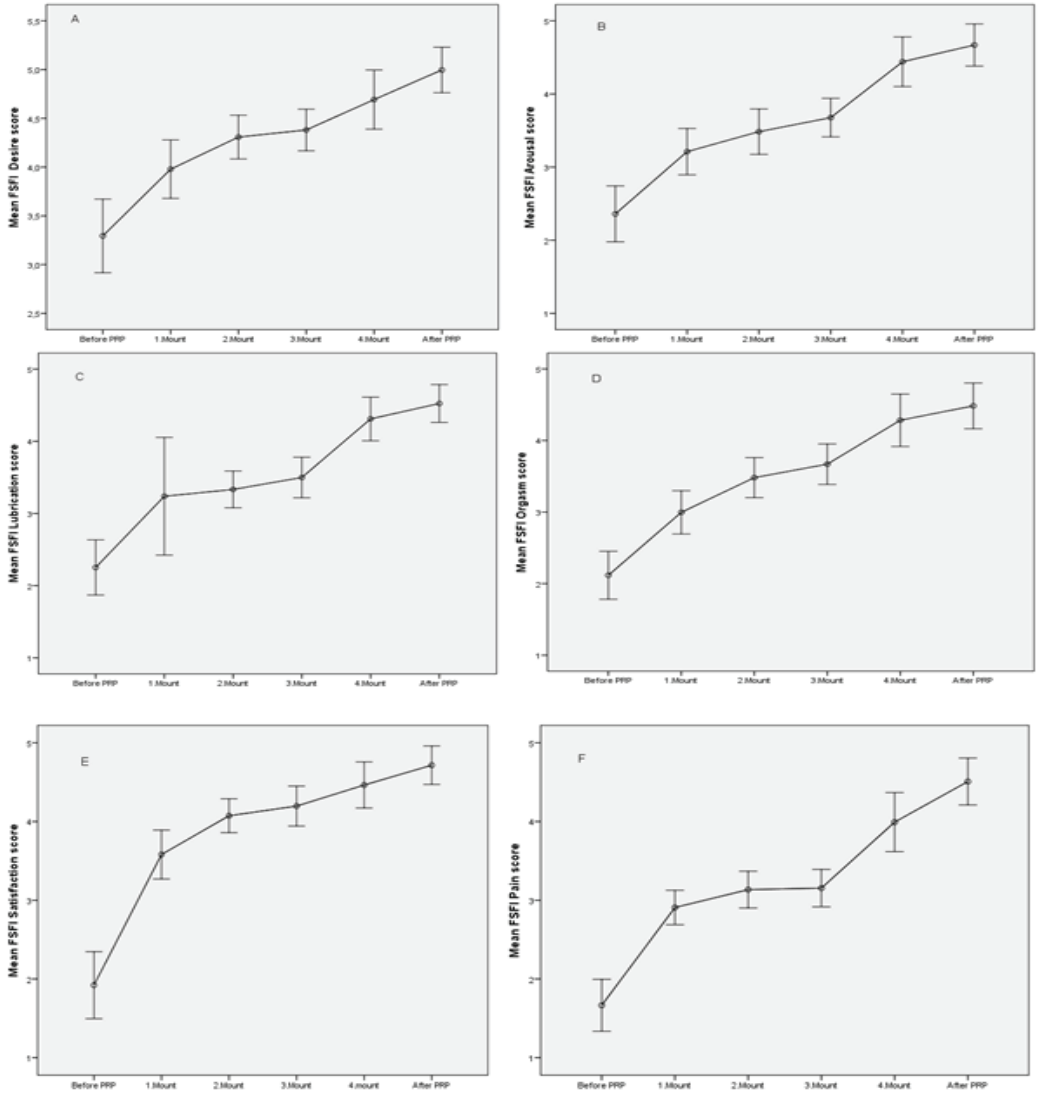

Figure 3. Graphs of mean Female Sexual Function Index (FSFI) subdomain scores at application times Mean FSFI desire score, B. Mean FSFI arousal score, C. Mean FSFI lubrication score, D. Mean FSFI orgasm score, E. Mean FSFI satisfaction score, F. Mean FSFI pain score. Bars are standard deviation. After PRP; 6th month checks are shown.

and is limited to case series, pilot studies, and case reports[2-3]. It has been used in atrophic diseases such as vaginal lichen sclerosis, stress urinary incontinence, episiotomy scars, and lubrication disorders[2,19]. PRP has been found to be effective in mesh erosion; PRP-coated mesh treatment has been revealed by *in vitro* and animal studies to result in better wound healing, increased synthesis of collagen 3, and neovascularization[20,21].

In studies investigating major motivators for genital aesthetic operations, notably labioplasty, it is seen that the expectation of an "increase in sexual function" is up to 50%[22,23]. In other words, to increase sexual functions, half of these patients have operations in which they may experience complications. In addition, the treatment of female sexual dysfunction is limited to psychotherapy and hormonal support in A-level[5]. For this reason, less invasive and low adverse-risk interventions may also be planned through good identification of the major motivators for patients whose only expectations are to increase sexual functions. From this point of view, laser, filler injections, and PRP are gaining popularity nowadays[3, 24-26]. It is also possible that in such minimally invasive methods, a positive effect is enhanced by providing the woman's sensitive focus to the vagina during sexual intercourse. The first use for sexual dysfunction in women was performed by Charles Runels under the name of O-Shot™[3]. Improvement of sexual functions was reported with PRP administration to the G-spot and glans clitoris. In this pilot study, PRP was administered to two regions, the clitoris and the anterior vaginal wall, into a space between the vagina and urethra most distal from bladder. However, the lack of a precise standardization of the location of the G-spot suggests that the administration area would result in a low response to

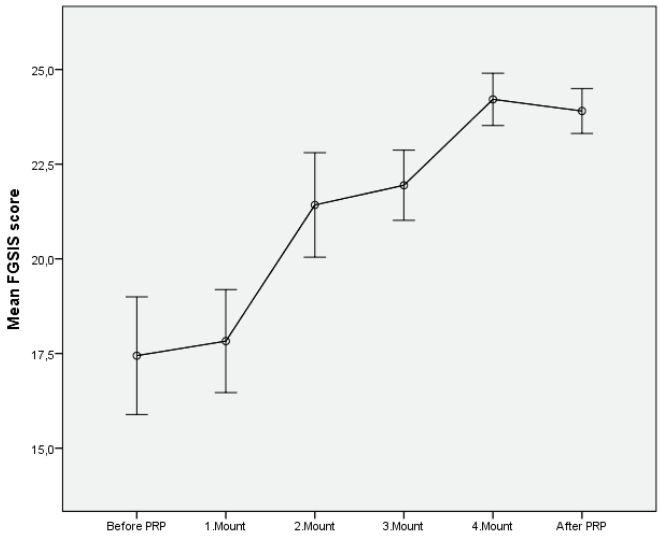

Figure 4. Graphs of mean the Female Genital Self-Image Scale Total score at application times. Bars are standard deviation. After platelet-rich plasma; 6th month checks are shown.

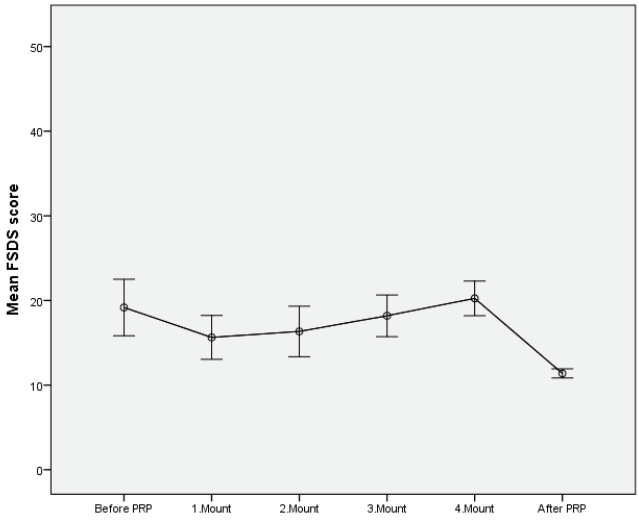

Figure 5. Graphs of Mean the Female Sexual Distress scale-revised Total score at application times. Bars are standard deviation. After PRP; 6th month checks are shown.

PRP: Platelet-rich plasma

a single injection.

In a human study, significantly increased density of nerves and microvessels in the distal one-third of the anterior vaginal wall were seen[9]. The anterior wall, compared with the posterior wall and the distal part of the anterior, has been shown to have more nerves immunohistochemically[7]. In a cadaver study, it was found that the second one-fifth partition of the distal anterior wall had significantly richer innervation than the surrounding areas[8].

The name G-spot, G-spot neurovascular complex, anterior wall erogenous complex, clitorovaginal-urethrovaginal complex, or whichever term it is called, is the female distal anterior vaginal wall, which is known to be more sensitive. The response of the distal anterior vagina, which is more sensitive, to penis-vagina penetration is higher[6,10,27]. Based on this, we planned to adminster our injections more commonly in the distal anterior one-third vagina region. We administered five injections in the vaginal wall. In previous studies, PPP has been shown to be as effective as PRP and to trigger angiogenesis and wound healing[28,29]. After centrifugation, we used the plasma part of the poor platelet in order to be able to benefit from the growth factors within it and due to our wide range of applications. It is known that increased blood flow through the clitoris is correlated with improved sexual function in women[30]. Accordingly, we administered four injections to the clitoris and its surroundings. There is no consensus in the literature about how many times and how often PRP administrations should be performed. Runels et al. made one single application for sexual dysfunction and they expressed positive results. In our study, we performed four administrations, but we found a significant change in total and all subdomains of FSFI after the first application. As the repetitions of the application increased, the acceleration of the positive effect decreased, but the increase continued. Therefore, the frequency of PRP and the number of repetitions can be planned according to the patient's condition.

In our study, FGSIS scores were found significantly lower than the mean values in the original study. This may be due to the fact that the selected population were patients with sexual dysfunction because the positive correlation between FSFI and FGSIS is known[12,17].

In our study, a significant decrease was observed in FSDS-R scores with the fourth administration. This shows that the administration results in a decrease in sexual distress. However, a negligible increase was observed in sexual distress after the first application until the third application. This may be the result of anxiety caused by expectation.

PGI-I scores, which we applied in the 6th month in order to evaluate the satisfaction of the patients, were found to be high, thereby supporting the positive increase in the survey scores.

To date, no adverse effect have been reported in vaginal applications in the literature. This is explained by the fact that the contents of PRP are from the patient's own body. In our study, no adverse effects of the administration were observed in any patients.

Study Limitations

The small number of studies in the literature on PRP and sexual dysfunction and the high number of patients in our study compared with other studies are strengths of our study. Furthermore, as far as we know, no similar study evaluating the efficacy of PRP in terms of orgasm is available in the literature. The lack of randomization and the lack of a control group to be performed with a placeboand retrospective design are the limitations of the study. Sexual dysfunction of partners in female sexual dysfunction is very important and the fact that this has

not been evaluated objectively another limitation of our study.

Conclusion

PRP is a minimally invasive method, which is easy to apply and fast, and has almost no adverse effects owing to it being autologous. Administration to the lower anterior vaginal wall may improve female sexuality with high satisfaction.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.
Authorship Contributions
Surgical and Medical Practices: G.S., A.E.K., E.K., E.Ç., Concept: G.S., A.E.K., E.K., E.Ç., Design:
G.S., A.E.K., E.K., E.Ç.,Data Collection or Processing: G.S., A.E.K., E.K., E.Ç., Analysis or Interpretation: G.S., A.E.K., E.K., E.Ç., Literature Search: G.S., A.E.K., E.K., E.Ç., Writing: G.S., A.E.K., E.K., E.Ç.
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Cervix human papilloma virus positivity: Does it cause sexual dysfunction?

Serviks human papilloma virüsü pozitifliği: Cinsel işlev bozukluğuna neden olur mu?

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Abstract

Objective: To investigate whether testing positive for human papilloma virus (HPV) in cervical screening has an impact on female sexual functioning.

Materials and Methods: This study was designed as a single-center, prospective, descriptive-cross-sectional study and 300 women who received HPV testing in our hospital [HPV-positive (n=187) or HPV-negative (n=113)]. The Arizona Sexual Experiences (ASEX) scale and Female Sexual Functioning index (FSFI) were administered to study participants during face-to-face interviews.

Results: No significant differences were found between women who were HPV-positive and HPV-negative in sexual functions as assessed using the ASEX and FSFI scales (p=0.343 and p=0.604, respectively). In addition, the analyses addressing whether sexual functioning was affected by a positive test result, at diagnosis or during the follow-up (before 2 weeks, 2 weeks-1 month, 1-3 months, 3-6 months, 6 months-1 year and over 1 year) revealed no significant differences between HPV-positive and HPV-negative women in sexual functioning (p>0.05). Sexual dysfunction was less common in married women than in the ASEX scale (p=0.03), and this difference was not detected when the FSFI scale was applied. The incidence of dysfunction was more frequent in working women than in retirees (p=0.006, p=0.01).

Conclusion: Educational attainment, socioeconomic status, age, employment status, and marital status were found to have statistically significant effects on sexual functioning. Sexual functioning was affected by neither HPV test results (positive/negative) nor time from diagnosis.

Keywords: Sexual dysfunction, physiological, human papilloma virus, cervix

Öz

Amaç: Çalışmadaki amacımız servikal human papilloma virüsler (HPV) taramasında pozitif sonuç alan hastalarda cinsel fonksiyonlarda değişiklik olup olmadığını araştırmaktır.

Gereç ve Yöntemler: Tek merkezli prospektif, tanımlayıcı-kesitsel olarak planlanan bu çalışma HPV testi yapılan 300 kadın hasta rastgele seçildi [HPV pozitif (n=187) ve HPV negatif (n=113)]. Yüz yüze görüşülerek Arizona Cinsel Yaşantılar Ölçeği (ACY) ve Kadın Cinsel İşlev Ölçeği (FSFI) uygulandı.

Bulgular: HPV pozitif ve negatif hastalar ile cinsel fonksiyonlar arasındaki ilişki açısından bakıldığında ACY ve FSFI ölçeklerinde anlamlı bir farklılık olmadığı izlenmiştir (p=0,343, 0,604). Ayrıca HPV tanısı alması anında ve takip süresince seksüel fonksiyonların etkilenip etkilenmemesine bakıldığında (tanıdan sonraki ilk 2 haftada, 2 hafta-1 ay, 1-3 ay, 3-6 ay, 6-12 ay ve 1 yıldan fazla) anlamlı bir farklılık bulunmamıştır (p>0,05).

Evli kadınlarda ACY ölçeğine göre cinsel fonksiyon bozukluğu (CFB) daha az görülmektedir (p=0,03), bu fark FSFI ölçeği uygulandığında saptanmamıştır. Çalışan kadınlarda CFB görülme oranı çalışmayan ve emeklilere göre daha sıktır (p=0,006, 0,01).

Sonuç: Eğitim düzeyi, sosyo-ekonomik durum, yaş, işte çalışıyor olmak ve medeni durumun cinsel fonksiyon üzerine istatistiksel olarak anlamlı olarak bulunmuştur. HPV tanısının pozitif ya da negatif olması ve HPV pozitif tanısı alan hastalarda tanı süresinin cinsel fonksiyonlar üzerine etkisi saptanmamıştır.

Anahtar Kelimeler: Cinsel fonksiyon bozukluğu, fizyolojik, HPV, serviks

PRECIS: Human papilloma virus results of the patients does not related to sexual dysfunction.

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Introduction

Female sexual function is defined as "the harmony in mind, senses and the individual's body, which leads to the achievement of the personality, communication and love" (World Health Organization 2006)[1]. Female sexual dysfunction is described as libido abnormalities, stimulation and orgasm problems, along with sexual pain[2].

In Turkey, the prevalence of sexual dysfunction in women has been reported to range between 46.9% and 48.3%[3,4]. Sexual function is affected by many factors such as menstruation, pregnancy, lactation, anogenital lesions, cancer, chronic systemic diseases, infertility, any conditions that might lead to sexual dysfunction including vaginismus, vaginal atrophy, vaginal stenosis, active vaginitis, hymenal stenosis, depression, use of any medicines, and alcohol and/or any chemical substance addiction[5-7].

Along with the changes in human sexual response model from linear to Basson's circular model[8], the definition of sexual dysfunction was also changed in the diagnostic and statistical manual of mental disorders-fifth edition criteria. The classification was made simpler by reducing categories into three sections. Female hypoactive desire dysfunction, and female arousal dysfunction were merged into a single syndrome called sexual interest/arousal disorder. Similarly, the formerly separate dyspareunia and vaginismus are now called genitopelvic pain/penetration disorder. Female orgasmic disorder remains in place[9].

Human papilloma virus (HPV) is the most prevalent sexually transmitted disease worldwide[10]. The transmission of HPV may also occur through intimate contact without intercourse. An HPV screening program has been conducted in Turkey since 2009. Owing to these screening programs, HPV can also be diagnosed in asymptomatic women. Being an HPV carrier and having anogenital condyloma have been associated with anxiety, depression, and sexual dysfunction in previous studies[11,12]. Women who tested positive for HPV may experience feelings of guilt, sadness, stigma, and embarrassment, which make them more concerned about sexual contact[13]. The aim of this study was to investigate sociodemographic factors affecting female dysfunction along with HPV screening results.

Materials and Methods

This study was designed as a single-center, prospective, descriptive-cross-sectional study, and was approved by the Dr. Lütfü Kırdar Training and Research Hospital Local Ethics Committee (decision no: 2019/514/148/24). Women who received HPV screening in our hospital between August 1st, 2017, and November 1st, 2017, were included in the study. The Arizona Sexual Experiences (ASEX) scale and the Female Sexual Function Index (FSFI) were administered to study participants during face-to-face interviews at diagnosis, before 2 weeks, 2 weeks-1 month, 1-3 months, 3-6 months, 6 months-1 year, and after 1 year. Each participant provided written informed consent. A total of 345 women aged 18 to 70 years who were referred for routine gynecologic exams with normal cervical smear results were included in the study. The exclusion criteria were pregnancy; lactation; anogenital lesions; cancer; chronic systemic diseases; infertility; any conditions that might lead to sexual dysfunction including vaginismus, vaginal atrophy, vaginal stenosis, active vaginitis, and hymenal stenosis; depression; use of any medicines; and alcohol and/or any chemical substance addiction. With all these evaluations, 300 patients were included and 45 patients were excluded from the study. The enrollment of the study was showed in Figure 1. The FSFI was developed by Rosen et al.[14] in 2000 to assess female sexual functioning. The validity and reliability of the Turkish version of the questionnaire has been established in a validation study[15]. It is a 19-item inventory comprising six domains: desire, arousal, lubrication, orgasm, satisfaction and pain. This questionnaire reflects sexual functioning over the past one month based on subscores from six subscales and the FSFI total score. Subscale scores and the FSFI total score are calculated according to a scoring system developed by the investigators who developed the questionnaire. Domain scores are calculated by summing individual items in a given subscale and multiplying the sum by corresponding domain factor indicated in the relevant table, and the overall FSFI score is calculated by summing all subscale scores. In this study, response options were provided and individual subscale scores were calculated on a Likert-type response scale. A total score less than 26.55 indicates risk for sexual dysfunction[16]. All questionnaires were reviewed in order to identify any possible inconsistency.

ASEX is a self-reported questionnaire that was developed by McGahuey et al. [17] to assess changes in sexual functioning and sexual dysfunction in patients on psychotropic medications. The validity and reliability of the Turkish version of the questionnaire has been established in a study conducted by Soykan. Female and male versions of ASEX are available. It is a five-item scale and items quantify sex drive, psychological arousal, physiological arousal, ability to reach orgasm, and satisfaction from orgasm, respectively. Each item is rated from 1 to 6 with possible total scores ranging from 5 to 30, sexual dysfunction is defined as total scores of 19 or more, or 5 or more on any item, or 4 or more on three items and strongly correlates with clinically defined sexual dysfunction[18,19]. Sociodemographic data including age, educational attainment, marital status, household income, and number of children were recorded. Etiologic factors related to FSD were investigated via the ASEX and FSFI questionnaires. Also, the questionnaire subscores of the patients were investigated in terms of HPV screening results. The patients were followed up in order to clarify the effect that could arise afterwards.

Statistical Analysis

The SPPS 20.0 software (IBM Corp. Released in 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM

Corp.) was used to analyze the study data. Data were presented as mean ± standard deviation, median (minimum-maximum), percentages, and frequency of variables. Repeated measures of analysis of variance (ANOVA) was analyzed using Mauchy's Sphericity test and Box's test of equality of covariance matrices. For comparisons of means, repeated measures ANOVA was used. If parametric tests (factorial design for repeated measures analysis) did not provide the preconditions, Greenhouse-Geisser (1959) correction or Huynh-Feldt (1976) correction or the Friedman test was used for corrections to the degrees of freedom. The corrected Bonferroni test was used for multiple comparisons. Normality and homogeneity of variances were prerequisites to analyze variables using the Shapiro-Wilk and Levene tests. In the analysis of data, the independent Samples t- test (Student's t-test) was used in comparisons between two independent groups, if prerequisites were met, and the Mann-Whitney U test was used if prerequisites were not met. In comparisons among three or more groups, One-way ANOVA and Tukey's honestly significant difference multiple comparisons test were used if prerequisites were met, and the Kruskal-Wallis or Bonferroni-Dunn's multiple comparisons tests were used if prerequisites were not met. Fisher's exact test and the chi-square test was used to analyze categorical data. When the expected frequencies were less than 20%, the Monte Carlo simulation method was used to include these frequencies in the analysis. The statistical significance level for these tests was set at p values of <0.05 and <0.01.

Results

A total of 300 women who met the inclusion criteria were included in the study. Cervical cytology samples were obtained. In the follow-up, results were explained to the patients and the ASEX and FSFI scales were administered to participants during this visit and follow-up visits at week 2, week 4, month 3, month 6 and month 12, thereafter. Study participants were stratified based on sociodemographic characteristics and the results of cervical cytology for HPV. The mean age of participants was 42 (range, 22-70) years. Sociodemographic Characteristics scale scores and smear results for HPV are summarized in Table 1.

The assessment of factors affecting total ASEX scale scores of the participants revealed that being aged 45 years and older (p=0.016), marital status (p=0.03), being employed (p=0.01),

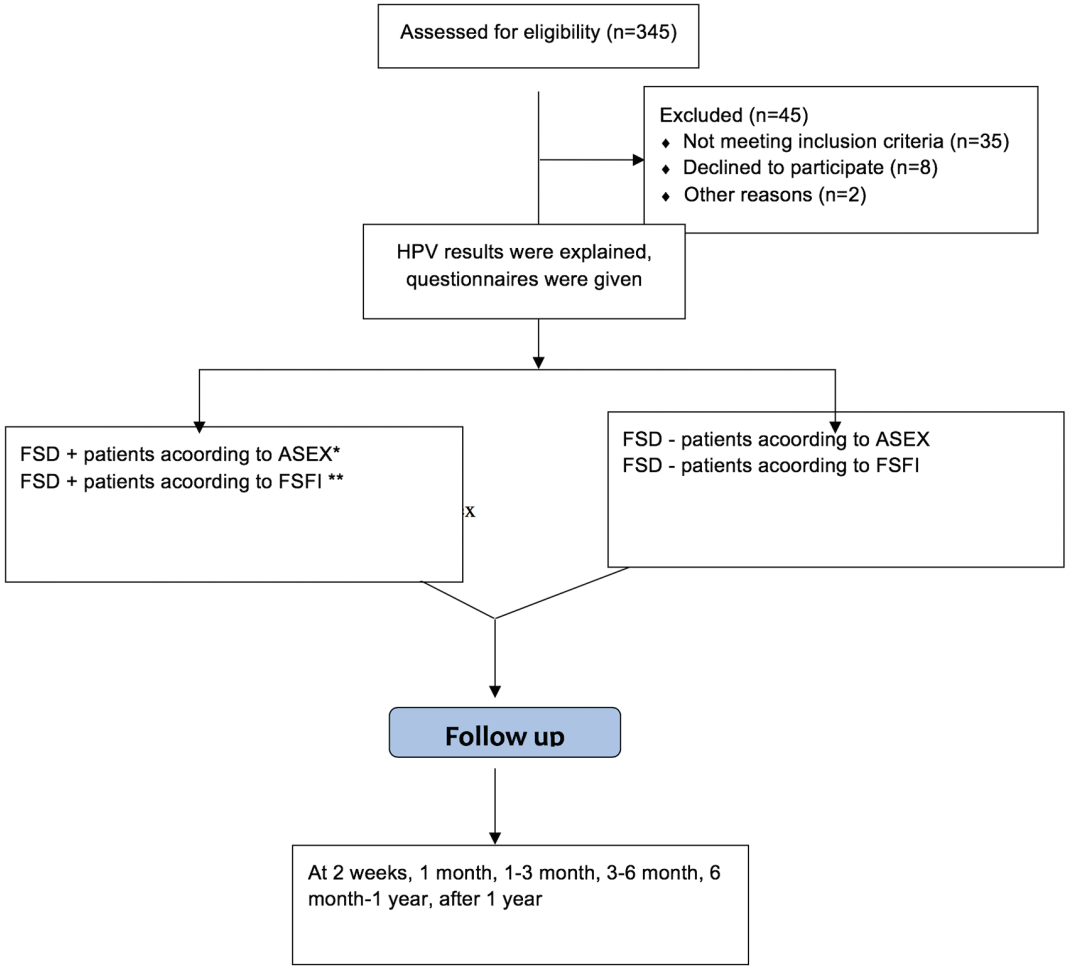

Figure 1. Follow-chart of enrollment
FSD: Functional sexual dysfunction, FSFI: Female Sexual Function index, ASEX: Arizona Sexual Experiances

parity (p=0.011), and low income and high income levels (compared to middle income levels) (p=0.023) significantly increased the prevalence of sexual dysfunction (Table 2). Testing positive for HPV and educational attainment were not found to have statistically significant effects on ASEX scores. The assessment of the effects of sociodemographic characteristics and testing positive for HPV on FSFI revealed that FSFI scores were significantly lower and sexual dysfunction was more common among working women (p=0.006). Age was found to significantly affect total FSFI scores and sexual dysfunction was more common among women aged 20 to 45 years than in women aged over 45 years (p=0.006). In the assessment of participants who tested positive for HPV, total FSFI scores were significantly higher in parous women compared with non-parous women (p=0.027) (Table 2). Income level, educational attainment, and marital status did not significantly affect FSFI scale scores.

The assessments on whether testing positive affected FSFI subscores revealed no differences between women who were HPV-positive and HPV-negative in terms of desire (p=0.670), arousal (p=0.670), lubrication (p=0.490), orgasm (p=0.880), satisfaction (p=0.850), and pain (p=0.380) subscale scores (Table 3).

The comparisons between ASEX scores or FSFI total/subscores at diagnosis, week 2, week 4, month 3, month 6 and month 12 time points revealed that neither ASEX scores nor FSFI total/subscores differed significantly with time from diagnosis of HPV (Table 4).

Table 1. Socio-demographic and maternal characteristics of participants

Variables	Mean ± SD	Median (minimum-maximum)
Age (years)	42.19±9.29	42 (22-70)
FSFI score	18.33±7.88	20.6 (2.40-34.40)
Arizona score	11.58±8.05	12 (0-30)
Education		
Illiterate	6	2
Primary	107	35.7
Secondary	56	18.7
High school	72	24
University	59	19.7
Marital status		
Married	231	77
Single	17	5.7
Widow	52	17.3
Parity		
Nulliparous	37	12.3
Multiparous	263	87.7
Employment		
Unemployed	132	44
Retired	147	49
Unemployed	21	7
Financial status (Turkish lira)		
<1500	105	35
1500-4500	145	48.3
>4500	50	16.7
HPV results		
HPV +	187	62.3
HPV -	113	37.7

HPV: Human papilloma virus, FSFI: Female Sexual Function index, SD: Standard deviation

Discussion

The HPV carrier incidence was 62.3% in our population, which is much higher than in older studies (17.9%), which might be associated with this study being conducted in a tertiary center. Also, previous studies were conducted in a different region of Turkey and 8 years ago[20]. Demir et al.[21] reported an HPV incidence of 31.8% among women aged 25-29 years and a decrease with age. Unlike other studies, this study could not demonstrate any potential effects of a diagnosis of HPV on sexual functioning. Furthermore, time from diagnosis had no effects on sexual functioning. Unlike our study, Ferenidou et al.[22] concluded that women who were diagnosed as having HPV experienced negative feelings and a reduction in sexual desire. According to the results of a study conducted by McCaffery et al.[23], testing positive for HPV might lead to anxiety, and necessary education should be provided to HPV-positive women after sharing test results. The results in our studies may be explained by the exclusion of women with anogenital lesions and abnormal Pap-smear results at screening. In addition, this study was the first and single study investigating the effects of a diagnosis of HPV and time from diagnosis on sexual functioning, and the differences in the results compared with studies conducted abroad might be explained by cultural differences. Psychosocial effects of HPV-linked diseases and abnormal cytology results have been demonstrated in previous studies[24]. Previous studies showed that even positive screen results for HPV might have psychosocial consequences. These effects could cause anxiety, stress or reluctance to engage in sexual activity. Independently from cervical cytology results, it has been demonstrated that a positive HPV test alone might make women feel bad about sexual relationships[25]. In our study group, sexual dysfunction scores at the time of detection of HPV positivity were examined and these scores were compared. However, it would be more

Table 2. Relationship between sociodemographic factors and human papilloma virus results of the patients and functional sexual dysfunction according to Female Sexual Function index and ARIZONA scores

		*FSFI scores		p	**ARIZONA scores		p
		***FSD positive n, %	FSD negative n, %		FSD negative n, %	FSD positive n, %	
Age (years)	20-45	187 (69)	14 (45.2)	0.006	173 (70)	28 (52.8)	0.016
	>45	82 (30.5)	17 (54.8)		74 (30)	25 (47.2)	
Education	Illiterate	5 (1.9)	1 (3.2)	0.183	5 (2)	1 (1.9)	0.091
	Primary	91 (33.8)	16 (51.6)		81 (32.8)	26 (49.1)	
	Secondary	50 (18.6)	6 (19.4)		49 (19.8)	7 (13.2)	
	High-school	66 (24.5)	6 (19.4)		58 (23.5)	14 (26.4)	
	University	57 (21.2)	2 (6.5)		54 (21.9)	5 (9.4)	
Relationship status	Married	202 (75.1)	29 (93.5)	0.062	183 (74.1)	48 (90.6)	0.03
	Single	16 (5.9)	1 (3.2)		15 (6.1)	2 (3.8)	
	Widow	51 (19.0)	1 (3.2)		49 (19.8)	3 (5.7)	
Employment	Working	124 (46.1)	8 (25.8)	0.006	116 (47)	16 (30.2)	0.01
	Unemployed	130 (48.3)	17 (54.8)		118 (47.8)	29 (54.7)	
	Retired	15 (5.6)	6 (19.4)		13 (5.3)	8 (15.1)	
Parity	Nulliparous	37 (13.8)	0 (0)	0.027	36 (14.6)	1 (1.9)	0.011
	Multiparous	232 (86.2)	31 (100)		211 (85.4)	52 (98.1)	
HPV results	HPV +	169 (62.8)	18 (58.1)	0.604	157 (63.6)	30 (56.6)	0.343
	HPV -	100 (37.2)	13 (41.9)		90 (36.4)	23 (43.4)	
Financial status (Turkish lira)	<1500	95 (35.3)	10 (32.3)	0.179	90 (36.4)	15 (28.3)	0.023
	1500-4500	126 (46.8)	19 (61.3)		111 (44.9)	34 (64.2)	
	>4500	48 (17.8)	2 (6.5)		46 (18.6)	4 (7.5)	

*FSFI: Female Sexual Function index, **Arizona Sexual Experiences scale, ***FSD: Functional sexual dysfunction, HPV: Human papilloma virus

meaningful to compare the scores of the same patient before the HPV test and the scores after receiving the HPV test results. This is the most important limitation of our research.

Female sexuality has been increasingly investigated over recent years. New questionnaires and algorithms have been developed to ensure the objectivity of assessments[26,27]. The FSFI is widely used to investigate female sexual functioning and received a wide acceptance; the validity of the Turkish version has been established[28]. In this study we also used the ASEX questionnaire in order to have more accurate results.

Previous studies investigated the associations between advanced age and sexual functioning and revealed similar rates of sexual problems between older women and younger women who were referred for routine gynecologic exams[29]. It has been reported that menopausal status has no negative impact on sexual functioning; however, stress, previous sexual experiences, and general health status are more important determinants of sexual health[30]. In this study, we also observed that sexual functioning was better in older women compared with younger women. Reduced responsibilities and mother and child dependency could explain this observation. The increased prevalence of sexual dysfunction among women with children

Table 3. Female Sexual Function index and Arizona comparative scores among women with and without human papilloma virus

Questionnaire	Mean scores ± SD		
	HPV +	HPV -	p
Total Arizona score	11.25±8.1	12.12±7.96	0.360
Desire	4.18±1.25	4.25±1.3	0.670
Arousal	2.69±1.97	2.85±1.86	0.490
Lubrication	2.9±1.7	2.95±1.57	0.810
Orgasm	2.65±1.58	2.67±1.46	0.880
Satisfaction	2.06±1.64	2.02±1.47	0.850
Pain	3.65±2.36	3.9±2.31	0.380
Total FSFI score	18.14±8.07	18.65±7.57	0.590

FSFI: Female Sexual Function index, HPV: Human papilloma virus, SD: Standard deviation

Table 4. Comparison of Female Sexual Function index and Arizona scores in time after testing positive for human papilloma virus

Questionnaire	<2 weeks Mean ± SD	2 weeks-1 month Mean ± SD	1-3 months Mean ± SD	3-6 months Mean ± SD	6 months-1 year Mean ± SD	>1 year Mean ± SD	p
Total Arizona score	14.29±7.55	9.36±9.23	11.3±7.57	11.14±8.86	10.77±7.88	11.47±7.14	0.488
Total FSFI score	21.41±7.08	15.62±9.92	18.72±7.24	17.73±8.62	17.53±8.02	18.35±6.8	0.447
Desire	4.09±1.34	4.54±1.09	4.05±1.23	4.42±1.47	4.42±1.47	4.14±1.21	0.334
Arousal	3.43±1.88	2.14±2.24	2.88±1.89	2.65±2.15	2.58±1.9	2.45±1.46	0.336
Lubrication	3.5±1.29	2.22±2.03	3.08±1.54	2.7±1.73	2.85±1.79	3.22±1.55	0.425
Orgasm	3.28±1.29	2.19±1.97	2.85±1.48	2.44±1.61	2.45±1.61	2.76±1.31	0.117
Satisfaction	3.05±1.6	1.86±2.16	2.02±1.39	2.01±1.71	1.87±1.46	1.77±1.35	0.554
Pain	4.08±2.07	2.67±2.57	3.84±2.23	3.51±2.43	3.51±2.43	3.64±2.42	0.221

FSFI: Female Sexual Function index, SD: Standard deviation, HPV: Human papilloma virus

compared with woman without children also provides further support to this assumption. In the literature, several studies reported that the first delivery and breastfeeding might cause sexual dysfunction[31]. A study assessing postpartum sexual functioning was conducted in the Netherlands and reported that older age at delivery was related to better sexual functioning[32]. In the literature, no association between educational attainment and sexual functioning has been clearly established[33]. In our study, educational attainment had no significant effects on ASEX total scores and FSFI total scores. In a study conducted by Laumann et al.[34], the prevalence of sexual dysfunction and possible predictors of sexual dysfunction were investigated and a negative correlation was found between higher educational attainment levels (college graduates) and the prevalence of sexual dysfunction. In our study, no significant association was found. These results may be explained by the fact that sex education is not included in the school curriculum in our country and any information is hearsay or obtained by individuals on their own. A literature search on the effects of income levels on sexual functioning revealed that several publications reported an association between lower income levels and a higher prevalence of sexual dysfunction, whereas others reported the opposite[35]. In this study, sexual dysfunction occurred less in the middle income group than in the other income groups. The assessment of income levels in combination with other variables indicated that sexual dysfunction was more likely among those with pre-existing depression and/or lower educational attainment, in addition to having a low income level. In previous studies, sexual dysfunction has been reported in more than one-third of women with more than 11 years of schooling[36]. However, there are also contradictory studies in the literature[37]. It is possible that we could not clearly demonstrate such differences because our population was not homogenous in terms of income levels. These differences might be clearly demonstrated in studies with larger and homogenous study samples. The effects of employment status on sexual dysfunction remained unclear in a recent study conducted in 2018, whereas partner's unemployment was suggested as a risk factor[38]. Sexual dysfunction was more prevalent in working women in our study. However, the nature of work and working hours were not investigated in our study, and potential effects of employment status on sexual functioning may be more clearly defined by taking these factors into consideration.

This study is important because it is the first to investigate the effects of testing positive for HPV on sexual functioning in Turkish woman over an extended follow-up period. Furthermore, bias was avoided by using two different questionnaires.

Conclusion

Educational attainment, socioeconomic status, age, employment status, and marital status were found to have statistically significant effects on sexual functioning. Sexual functioning was affected by neither HPV test results (positive/negative) nor time from diagnosis.

Ethics

Ethics Committee Approval: This study was designed as a single-center, prospective, descriptive-cross-sectional study, and was approved by the local Ethics Committee (Dr. Lütfi Kırdar Training and Research Hospital, decision no: 2019/514/148/24).

Peer-review: Externally peer-reviewed.

Informed Consent: Each participant provided written informed consent.

Authorship Contributions

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

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Evaluation of the prevalence of sexually transmitted bacterial pathogens in Northern Cyprus by nucleic acid amplification tests, and investigation of the relationship between these pathogens and cervicitis

Kuzey Kıbrıs'ta cinsel yolla bulaşan bakteriyel patojenlerin prevelansının nükleik asit amplifikasyon testleri ile incelenmesi ve bu patojenlerin servisitle olan ilişkilerinin araştırılması

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Abstract

Objective: To evaluate the prevalence of pathogens, *Chlamydia trachomatis, Neisseria gonorrhea* and *Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum,* and *Ureaplasma parvum* in women via multiplex-polymerase chain reaction (PCR)-deoxyribonucleic acid (DNA).

Materials and Methods: Cervical swabs of 273 women in reproductive age who underwent gynecologic examination in our outpatient clinic were evaluated using the multiplex-PCR-DNA method. The presence of cervicitis, contraceptive methods, marital status, and the number of partners were evaluated.

Results: One hundred six (39%) of the 273 women had at least one bacterium, 25 women (9.8%) had two bacteria, and three women (1%) had three bacteria. *U. urealyticum* was the most frequently encountered bacterium (13.9%), followed by *M. hominis* (12.8%), *U. parvum* (12.4%), *C. trachomatis* (5.4%), *M. genitalium* (2.9%), *N. gonorrhea* (2.5%), and *T. vaginalis* (0.3%). Bacterial infection was detected more frequently in women aged <25 years, single, who had multiple partners, and clinically diagnosed with cervicitis. The cervicitis rate was 39% in our study. M. genitalium was significantly more frequent in women with cervicitis than in women without cervicitis (5.6 vs. 1.2%, p=0.005). *C. trachomatis* and *N. gonorrhea*, which are often associated with cervicitis, were comparable in women with and without cervicitis.

Conclusion: Women with clinically diagnosed cervicitis or even with a normal-appearing cervix should be tested using multiplex-real-time PCR-nucleic-acid-amplification tests on suspicion of such an infection. *M. genitalium* is an emerging bacterial agent for cervicitis along with *C. trachomatis* and *N. gonorrhea.*

Keywords: Chlamydia, neisseria, trichomonas, mycoplasma, ureaplasma

Öz

Amaç: Kadınlarda multipleks-polimeraz zincir reaksiyonu (PCR)-deoksiribonükleik asit (DNA) ile *Chlamydia trachomatis, Neisseria gonorrhea* ve *Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum* ve *Ureaplasma parvum* patojenlerinin görülme sıklığını değerlendirmek ve klinik olarak servisit tanısı koyulan kadınlardaki bu bakterilerin rolünü incelemek.

Gereç ve Yöntemler: Polikliniğimizde jinekolojik muayeneye gelen üreme çağındaki 273 kadından alınan serviks sürüntüsü multipleks-PCR-DNA yöntemiyle incelendi. Servisit varlığı, kontraseptif yöntemler, evlilik durumu ve partner sayısı değerlendirildi.

Bulgular: İki yüz yetmiş üç kadından 106'sında (%39) en az bir bakteri tespit edilirken, 25 kadında (%9,8) iki bakteri, 3 kadında ise (%1) üç bakteri tespit edildi. *U. urealyticum* (%13,9) en sık tespit edilen bakteri olup, sıklık sırasına göre *M. hominis* (%12,8), *U. parvum* (%12,4), *C. trachomatis* (%5,4), *M. genitalium* (%2,9), *N. gonorrhea* (%2,5) ve *T. vaginalis* (%0,3) oranında saptandı. Bakteriyel infeksiyon 25 yaşın altında, bekar, birden fazla partneri olan ve klinik olarak servisit tanısı

PRECIS: To identify the possible risk factors for postpartum urinary retention.

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koyulan kadınlarda daha sık gözlendi. Çalışmamızda servisit oranı %39 olarak saptandı. *M. genitalium* servisit görülen kadınlarda servisit görülmeyen kadınlara kıyasla anlamlı olarak daha sık saptandı (%5,6'ya karşılık %1,2, p=0,005). Sıklıkla servisitle ilişkilendirilen *C. trachomatis* ve *N. gonorrhea*'nın görülme oranları benzer olarak saptandı.

Sonuç: Klinik olarak servisit tanısı koyulan kadınlarda veya enfeksiyon şüphesi varsa normal görünümdeki serviks varlığında bile multipleks-PCR-nükleik asit amplifikasyon testleri kullanılmalıdır. *M. genitalium*, *C. trachomatis* ve *N. gonorrhea*'nın ardından bakteriyel servisit etkeni olarak öne çıkmaktadır.

Anahtar Kelimeler: Klamidya, neisseria, trikomonas, mikoplazma, üroplazma

Introduction

Every year, more than 1 million people are infected with sexually transmitted diseases (STDs)[1]. *Chlamydia trachomatis, Neisseria gonorrhea* and *Trichomonas vaginalis* are the very well-known sexually transmissible pathogens, whereas *Mycoplasma genitalium* has recently gained importance in the pathogenesis of cervicitis[2]. These bacteria are either asymptomatic or present themselves with mild symptoms, which may easily be overlooked[1]. These bacterial STDs may lead to tubal infertility and extrauterine pregnancy as well as chronic pelvic pain, which is associated with a severe socioeconomic burden[1]. Besides *Mycoplasma hominis* and *Ureaplasma urealyticum, Ureaplasma parvum* may be commensally colonized in the cervix. However, some authors suggest that such colonization may be associated with poor obstetric outcome, postpartum sepsis, and neonatal infections[3]. The serologic diagnosis or traditional culture media may not be sufficient for diagnosis[2,4]. In some cases, the presence of multiple agents makes it even more difficult to diagnose the actual agents[4]. For that reason, due to their high sensitivity and specificity for the diagnosis of STDs, as well their ability to diagnose more than one pathogen at once, multiplex real-time polymerase chain reaction (PCR) nucleic acid amplification tests (NAAT) have gained popularity over conventional microbiologic culture methods[4-6]. In this study, we aimed to evaluate the prevalence of pathogens including *C. trachomatis, N. gonorrhea, T. vaginalis*, *M. hominis*, *M. genitalium*, *U. urealyticum* and *U. parvum* in women via multiplex PCR DNA tests, and to assess the role of these bacteria in women with clinically diagnosed cervicitis who were admitted to our outpatient clinic in Near East University for gynecologic examinations.

Material and Methods

In this study, the cervical swabs of 273 women in reproductive age who were admitted for gynecologic examinations with symptoms of vaginal discharge or who asked for a screening of sexually transmitted infections without any symptoms to the outpatient clinic of Near East University, Department of Obstetrics and Gynecology, between 2014 and 2016, were examined using the multiplex PCR DNA method. The results were retrospectively evaluated. The study was approved by the Ethics Committee of Near East University on March 31st, 2016 (number: of 2016/36-266). Informed and signed consent was obtained from all participants. Inclusion criteria were apparently healthy, sexually active women aged >18 years without pelvic pain or fewer, who were not pregnant, and had not received antibiotic recently for a gynecologic infection. Women with pelvic inflammatory disease (PID) were excluded from the study. All women had a gynecologic examination, and the presence of cervicitis, contraceptive methods, marital status, and the number of partners were documented.

Cervicitis was described as the presence of purulent or mucopurulent discharge and/or hyperemic, edematous and friable (bleeding even with a light touch of a cervical swab) cervix[7,8]. The cervical swabs from participants were taken by gynecologists using a single-use speculum with the manufacturer's kits and were sent to the genetic laboratory.

Nucleic Acid Isolation Procedure

Nucleic acid isolation was performed in accordance with the manufacturer's instructions (GeneAll RibospinTM vRD). Swab samples were obtained from the cervix and then transferred to the Medical Genetics Laboratory of Near East University Hospital. The following steps were performed: Centrifugation at 5000 rpm for 15 minutes, addition of buffer (VL, 500 µL), incubation for 10 min at 25 °C, addition of buffer (700 µL RB1), and vortexing. Preparation of the spin column. Removal of residual buffer by centrifugation of the mixture at 12,000 g. Addition of nuclease-free H_2O. Re-centrifugation at over 10,000 g for 60 seconds. The purified nucleic acid was kept at 4 °C for direct analysis and kept at -70 °C for subsequent analysis.

Polymerase Chain Reaction

PCR was conducted for detecting the STD panel. The fast track diagnostic urethritis plus real-time PCR kit was used for analysis, which examines *C. trachomatis, N. gonorrhea, T. vaginalis*, *M. hominis*, *M. genitalium*, *U. urealyticum,* and *U. parvum*. The DNA amplification reactions were performed using Qiagen Rotor-gene Q. After the DNA amplification, the results were interpreted according to the given fluorescence trace of the positive samples. The results were examined using the data supplied by the manufacturer.

Statistical Analysis

Continuous parametric variables are given as mean and standard deviation. Categorical variables are expressed as number or percentage. T-test or analysis of variance were used for the comparison of parametric variables. Categorical variables were compared using the chi-square (χ^2) test. Statistical calculations were performed using Statistical Package for Social Sciences (SPSS 15.0, Chicago, IL, USA). $P<0.05$ was accepted as significant.

Results

A total of 273 women were included in this study. The demographic and clinical features of the patients are given in Table 1. The mean age of the women was 31.03±9.20 years. The study group consisted mainly of married women (70%), with a single partner (85%); 39.5% of the women had cervicitis. One hundred six (39%) of the 273 women had at least one bacterium, 25 women (9.8%) had two bacteria, and 3 women (1%) had three bacteria. Among the 273 women, *U. urealyticum* was the most frequently encountered bacterium in the cervix (13.9%), followed by *M. hominis* (12.8%), *U. parvum* (12.4%), *C. trachomatis* (5.4%), *M. genitalium* (2.9%), *N. gonorrhea* (2.5%), and *T. vaginalis* (0.3%). The infection rates according to the age, marital status, number of partners, the presence of cervicitis, and type of contraceptive method are presented in Table 2. Bacterial infection was detected more frequently in women aged <25 years, those who were single, who had multiple partners, and clinically diagnosed with cervicitis. Bacterial infection was detected less frequently in women who used a condom as a contraceptive method. *M. hominis* was the most commonly seen bacterium in women aged under 25 years. *M. hominis* and *U. urealyticum* were significantly more common in women with multiple partners. The cervicitis rate

Table 1. The demographic and clinical features of the patients

	Number of women	percent (%)
Age groups		
<25 years	83	30.40
26-30 years	78	28.57
31-35 years	48	17.58
>36 years	64	23.44
Marital status		
Single	81	29.67
Married	192	70.33
Number of partners		
One	233	85.35
Multiple	40	14.65
Deliveries		
No	122	44.69
Yes	151	55.31
Vaginal discharge		
Absent	231	84.62
Present	42	15.38
Cervicitis		
absent	165	60.44
present	108	39.56
Contraceptive method		
Condom	17	6.23
Withdrawal (coitus interruptus)	160	58.61
Oral contraceptives	63	23.08
Intrauterine device	33	12.09

Chi-square test, p<0.05 was accepted as statistically significant

Table 2. The infection rates according to the age, marital status, number of partners, vaginal mucopurulent discharge, presence of cervicitis and type of contraceptive method

	No detectable bacteria		Bacteria positive		p
	n	%	n	%	
Age groups					
<25 years	42	50.60	41	49.40	0.09
26-30 years	50	64.10	28	35.90	
31-35 years	30	62.50	18	37.50	
>36 years	45	70.31	19	29.69	
Marital status					
Single	36	44.44	45	55.56	<0.001**
Married	131	68.23	61	31.77	
Number of partners					
One	155	66.52	78	33.48	<0.001**
Multiple	12	30.00	28	70.00	
Parity					
No	65	53.28	57	46.72	0.02*
Yes	102	67.55	49	32.45	
Complaint					
Asymptomatic	147	63.64	84	36.36	0.05*
Vaginal mucopurulent discharge	20	47.62	22	52.38	
Cervicitis					
Absent	110	66.67	55	33.33	0.02*
Present	57	52.78	51	47.22	
Contraceptive method					
Condom	16	94.12	1	5.88	0.03*
Withdrawal (coitus interruptus)	93	58.13	67	41.88	
Oral contraceptives	36	57.14	27	42.86	
Intrauterine device	22	66.67	11	33.33	

Chi-square test, p<0.05 was accepted as statistically significant

was 39% among the 273 women in our study. Among women with cervicitis, M. genitalium was significantly more frequent in women with cervicitis than in those without cervicitis (5.6% vs. 1.2% p<0.005). *C. trachomatis* and *N. gonorrhea,* which are often associated with cervicitis, were comparable in women with and without cervicitis. In 55 (33%) of 165 women with no clinical cervicitis, at least one bacterium was detected, and 15 (9%) women had at least one of the bacteria known to be associated with cervicitis, such as *C. trachomatis*, *N. gonorrhea*, *M. genitalium* or *T. vaginalis*. By contrast, among 108 women with clinical cervicitis, the rate of the bacteria known to be associated with cervicitis was 14.9% (9% vs. 14.9%; p=0.133). The rates of simultaneous infections with multiple bacteria were comparable between women with and without cervicitis (9.3% vs. 11.5%, p=0.565).

Discussion

In our study, 39% of women had at least one bacterium. Among the 273 women, *U. urealyticum* was the most frequently encountered bacterium in the cervix (13.9%), followed by *M. hominis* (12.8%), *U. parvum* (12.4%), which showed a balanced distribution. The detection rate of bacteria was reported to vary between 30.7 and 49% in previous screened populations[4,5]. Kim et al.[5] screened 799 Korean women and detected at least one bacterium in 49% of women. Contrary to our study, *U. parvum* was the most frequently (32.5%) found bacterium, followed by *U. urealyticum* (3.5%) and *M. hominis* (1%). Lee et al.[4] screened 304 women and detected bacteria in 36.5%, most frequently *U. urealyticum* (14.5%), followed by *M. hominis* (13.8%). In south Italy, Del Prete et al.[9] screened 1272 women and detected at least one bacterium in 30.7% of women. The most commonly detected bacterium was by far *U. parvum* (25.9%). In our study, we detected the bacterial colonization of *U. parvum* with a rate of 12.4%. Yamazaki et al.[10] reported high detection rates of *U. parvum* as 41.7%. Yamazaki et al.[10] suggested that the high prevalence of the latter two bacteria might be attributed to the region, culture, and tendency to nightlife. Camporiondo et al.[6] performed a screening study in 309 Italian women and detected no *C. trachomatis*, *M. genitalium* or N. *gonorrhea,* but *U. parvum* (28.8%), *M. hominis* (3.9%) and *U. urealyticum* (4.5%). McIver et al.[11] evaluated 175 sexually active Australian women and detected U. parvum (53%), *M. hominis* (7.4%), and *U. urealyticum* (3.4%) in descending order. Simultaneous infection rates with *U. parvum* + *M. hominis*, *U. urealyticum* + *M. hominis* and *U. urealyticum* + *U. parvum* were 7.4%, 1.1%, and 2.9%, respectively. In our study *U. urealyticum*, *M. hominis*, and *U. parvum* were among the most commonly detected bacteria in the cervix. They are accepted as genital commensalistic organisms and found in healthy women[10]. Routine screening and treatment of the latter three bacteria are controversial[10]. Some authors suggest that colonization with *U. urealyticum* and *U. parvum* in high density is associated with non-specific cervicitis[12], whereas others suggest that there is not enough evidence to suggest that these bacteria cause cervicitis or PID[13]. However, several studies in pregnant women showed that the presence of these bacteria in amniotic-fluid or membranes might be associated with preterm labor, preterm premature rupture of membranes (PPROM), and neonatal infections[14-24]. Abele-Horn et al.[21] showed that *U. urealyticum* was associated with preterm labor. Kataoka et al.[22] evaluated 877 pregnant women under 11 gestational weeks (GW) and detected the prevalence rates of *U. urealyticum, M. hominis*, and *U. parvum* as 52.0%, 11.2%, and 8.7%, respectively. Despite the higher prevalence of U. urealyticum in the latter study, *U. parvum* had a stronger association with late abortion and preterm labor compared with *U. urealyticum*[22]. One hundred eighty-four pregnancies complicated with preterm labor and PPROM were evaluated in a prospective study and coinfection with *M. hominis,* and *U. urealyticum* was shown to be associated with poorer pregnancy outcomes compared with infection with *U. urealyticum* alone[23]. In another study, vaginal *U. urealyticum* and *U. parvum* colonization were also shown to be associated with chorioamnionitis in pregnancies under 28 GW complicated with PPROM[24]. Rumyantseva et al.[25] recently evaluated 1773 women and observed that the isolation rates of *U. parvum* and *M. hominis* in women with bacterial vaginosis were significantly higher in women with altered vaginal microflora compared with women with normal vaginal flora. Chlamydia is known to be the most common STD[26,27]. It is one of the major organisms causing cervicitis and PID, even if it is asymptomatic. In our study, the prevalence of *C. trachomatis* was 5.4%, which was more frequent than other sexually transmissible bacteria such as *M. genitalium* (2.9%), *N. gonorrhea* (2.5%) and *T. vaginalis* (0.3%). Chlamydia prevalence, along with the prevalence of other STDs, may vary according to the age, race, region, and socioeconomic status[26,28]. The prevalence of chlamydia was reported as 0.6% in Australia[11], 2.6% in the Netherlands[29], 2.3% in China[30], and as high as 14.2% in South Africa[31]. The prevalence of chlamydia in the United States of America (USA) was reported to be 4.2% in the general population, but as high as 10% in Mexicans living in the USA (26). In a systematic review and meta-analysis, the prevalence of chlamydia in Europe and developed countries such as Canada, Australia, and New Zealand was reported as 3.0-5.3%[28], which is also concordant with the values in our study. In the present study, the prevalence of gonorrhea was 2.5%. Gonorrhea is the second most common sexually transmitted bacterial infection following chlamydia[26]. According to the World Health Organization report in 2012, the global gonorrhea prevalence varies between 0.3% and 1.7%[1]. In our study, the prevalence of gonorrhea was significantly over the global average. Nevertheless, the prevalence of STD may vary according to the country or even to the region in the same country. Kim et al.[5] evaluated 799 Korean women and detected no gonorrhea, whereas Lee et al.[4] evaluated 304 Korean women and detected gonorrhea in 3.3% of the screened population, which was even higher than

in our study group. Gaydos et al.[32] evaluated a group of 324 women comprising mainly young African-American women in Baltimore, USA, and detected the rate of gonorrhea as 4.6%. The high rates of gonorrhea in our study may be attributed to the presence of nightclubs in our region. In our study, the rate of *M. genitalium* infection was 2.9%. The global *M. genitalium* infection rate is reported as 1-6.2%[33]. Nevertheless, prevalence rates as high as 19.2% have also been reported[32]. Nowadays, since the widespread use of NAATs in recent years, *M. genitalium* counts as one of the most important bacteria, following *N. gonorrhea* and *C. trachomatis*, causing cervicitis[34]. The prevalence of *T. vaginalis* was 0.3% in our study. *T. vaginalis* is the most common non-viral sexually transmissible infection in the USA and may cause urethritis in men and women, and vaginitis and cervicitis in women[35]. It is hard to determine the true prevalence of *T. vaginalis* because the feedback is not so efficient as with other STD pathogens. According to the Centers for Disease Control and Prevention, the prevalence of *T. vaginalis* in non-Hispanic women in the USA is 1.8%[2]. Moreover, in hitherto literature, prevalences of *T. vaginalis* as low as 0.1% have been reported[5].

Cervicitis

In our study, the cervicitis rate was 39% among 273 women, which is similar to the 41% among 324 women reported by Gaydos et al.[32]. Gaydos et al.[32] detected that *C. trachomatis* and *M. genitalium* were associated with cervicitis; however, only *M. genitalium* had a significant association with cervicitis in multiple regression analysis. In our study, the *M. genitalium* infection rate was significantly elevated in women with clinical cervicitis compared with women without cervicitis, which supports recent data about the importance of *M. genitalium* as an emerging pathogen of cervicitis. The clinical diagnosis of cervicitis is not always suggestive for a sign of bacterial infection. It has been shown that no infectious pathogen is detectable in the majority of women with clinical cervicitis[36]. Nevertheless, *N. gonorrhea* and *C. trachomatis* have long since been reported to be the most frequent bacteria causing cervicitis, if a pathogen is detectable[37,38]. Along with *N. gonorrhea* and *C. trachomatis*, *M. genitalium*, *T. vaginalis*, and Herpes simplex virus, as well as *Gardnerella vaginalis*, may cause cervicitis[34]. In our study, there was no detectable bacterial pathogen with NAAT PCR DNA in 57 of 108 women (52%) with clinical cervicitis. At this point, it should be mentioned that etiologic factors of non-infectious cervicitis include chemical or physical trauma, vaginal douche, idiopathic inflammation of the cervix, and Behçet' disease[2]. In our study, in 55 (33%) of 165 women with no clinical cervicitis at least bacterium was detected, and fifteen (9%) women had at least one of the bacteria known to be associated with cervicitis, such as *C. trachomatis*, *N. gonorrhea*, *M. genitalium* or *T. vaginalis*. By contrast, among 108 women with clinical cervicitis, the rate of the bacteria known to be associated with cervicitis was 14.9%. The coinfection rates in women with and without cervicitis were comparable in our study, concordant with the findings of Gaydos et al.[32]. Studies about the detection rates of infectious agents causing vaginitis and cervicitis are summarized in Table 3[4-6,9,11,32,39]. In our study, it has been shown that multiplex real-time PCR-NAAT is beneficial for the demonstration of cervical bacterial colonization with commensal pathogens including *U. urealyticum*, *M. hominis*, and *U. parvum* or with pathogens that can cause cervicitis such as *C. trachomatis*, *N. gonorrhea*, and *M. genitalium*. The major limitation of these techniques is their high costs[40].

Conclusion

In our study the prevalence of sexually transmitted bacteria in the Northern Cyprus region was consistent with the literature. *M. genitalium* was detected to be more frequent in women with cervicitis than in women without cervicitis (5.6% vs. 1.2% p<0.005). *C. trachomatis* and *N. gonorrhea*, which are often associated with cervicitis, were comparable in women with and without cervicitis.

According to our study results, women either diagnosed with cervicitis or with a normal-appearing cervix should be tested with multiplex-real-time PCR-NAATs on suspicion of such an infection. There was no significant difference regarding simultaneous infection with multiple bacteria between women with and without cervicitis.

Table 3. Studies about the detection rates of infectious agents causing vaginitis and cervicitis

	Present study % (n=273)	Lee et al.[4] (n=304)	Kim et al.[5] (n= 799)	McIver et al.[11] (n=175)	Camporiondo et al.[6] (n=309)	Del Prete et al.[9] (n=1272)	Choe et al.[39] (n=201)	Gaydose et al.[32] (n=324)
U. urealyticum	13.9	14.1	7.6	6.1	4.5	5.1	40.3	-
M. hominis	12.8	13.8	9.9	13.7	3.9	5.9	14.9	-
U. parvum	12.4	-	42	57	28.8	24.9	52.7	-
C. trachomatis	5.4	3.0	1.1	0.6	-	1.8	4.0	11.1
M. genitalium	2.9	0.3	1.0	1.3	-	0.3	4.0	19.2
N. gonorrhoeae	2.5	3.3	0.0	-	-	0.1	1.5	4.6
T. vaginalis	0.3	2.0	0.1	4.0	1.3	1.4	1.0	15.3

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Near East University in 31.03.2016 with the number of 2016/36-266.
Informed Consent: Informed and signed consent was obtained from all participants.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: O.G., Data Collection or Processing: O.G., B.K., Analysis or Interpretation: O.G., A.B., E.Ö.B., Literature Search: O.G., M.S.S., Writing: O.G.
Conflict of Interest: No conflict of interest was declared by the authors.
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The potential of serum fetal DNA for early diagnosis of gestational trophoblastic disease

Gestasyonal trofoblastik hastalığın erken tanısında serum fetal DNA'nın potansiyeli

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Abstract

Objective: To study cell-free DNA (cfDNA) levels in patients with gestational trophoblastic disease (GTD) in order to test the hypothesis that cfDNA circulating in maternal plasma could provide early detection of GTD.

Materials and Methods: This study included 32 patients with GTD (complete mole and partial mole) and 30 non-GTD patients in the first trimester of pregnancy with no other medical problems. cfDNA levels in maternal serum were measured using polymerase chain reaction analysis on Y-chromosome–specific sequences.

Results: cfDNA was found as 327±367 pg on average in the control group and 600±535 pg in the GTD group. Within the GTD group, the partial mole group had an cfDNA average of 636±549 pg, and the complete mole group had an cfDNA average of 563±536 pg. Although there was a statistically significant difference between the GTD group and the control group in terms of cfDNA (p=0.02), there was no statistically significant difference between the complete mole group and the partial mole group (p=0.76).

Conclusion: Non-parametric analysis of covariance in terms of cfDNA in GTD was performed, thereby increasing its power and revealing a significant difference compared with the control group. This indicates that maternal peripheral bloodstream cfDNA monitoring might be significant in the early diagnosis of GTD.

Keywords: cffDNA, complete mole, gestational trophoblastic disease, partial mole, polymerase chain reaction

Öz

Amaç: Maternal plazmada dolaşan serum fetal DNA'nın (cfDNA) gestasyonel trofoblastik hastalığın (GTH) erken tanısında potansiyeli olduğunu tespit etmek için GTH'de cfDNA düzeylerini inceledik.

Gereç ve Yöntemler: Çalışmaya, gebeliğin ilk üç ayında diğer tıbbi problemleri olmayan 32 GTH hastası (komplet mol ve inkomplet mol) ve GTH olmayan 30 hasta dahil edildi. Maternal serumdaki cfDNA seviyeleri, Y-kromozomuna spesifik dizinlerde polimeraz zincir reaksiyonu analizi kullanılarak ölçüldü.

Bulgular: Kontrol grubunda cfDNA ortalama olarak 327±367 pg, GTH grubunda 600±535 pg olarak bulundu. GTH grubunda Inkomplet mol grubu cfDNA ortalaması 636±549 pg idi ve komplet mol grubu cfDNA ortalaması 563±536 pg idi. GTH grubu ile kontrol grubu arasında istatistiksel olarak anlamlı bir fark vardı (p=0,02). cfDNA açısından, komplet mol grubu ile inkomplet mol grubu arasında istatistiksel olarak anlamlı bir fark bulunmadı (p=0,76).

Sonuç: GTH'de cfDNA açısından kontrol grubuyla karşılaştırıldığında anlamlı bir fark ortaya çıktı. Bu maternal periferik kan akımı cfDNA izlemenin GTH'nin erken tanısında önemli olabileceğini göstermektedir.

Anahtar Kelimeler: cfDNA, komplet mol, gebelikte trofoblastik hastalık, inkomplet mol, polimeraz zincir reaksiyonu

Introduction

Gestational trophoblastic disease (GTD) refers to a spectrum of disorders that result from abnormal proliferation of the trophoblastic epithelium. These include premalignant disorders (hydatidiform moles) and malignant disorders (persistent/invasive gestational trophoblastic neoplasia, placental site

PRECIS: cfDNA in gestational trophoblastic disease.

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trophoblastic tumors, epithelioid trophoblastic tumors, and choriocarcinoma). Most patients present with abnormal beta-hCG levels and abnormal ultrasound. Laboratory evaluation and imaging of the chest, abdomen, and pelvis are recommended to determine the appropriate treatment and follow-up. Complete recovery is possible in patients who are diagnosed early and who receive adequate treatment in the course of the disease[1]. Free DNA is naked, double-stranded DNA independent of the cell, and it can be obtained from the circulatory system by means of plasma purification or serum samples[2]. In the last 30 years, the importance of a detailed study of free DNA has become more apparent[3,4]. Approximately 95% of the free DNA isolated from the mother during pregnancy belongs to the mother, and 5% belongs to the fetus, and this is called cell-free DNA (cfDNA). It has been shown that 1 mL of blood in a mother with a healthy pregnancy usually contains one fetal cell; this ratio increases in the majority of pathologic pregnancies[5,6]. Circulating fetal DNA has also been identified as a marker for assessing fetomaternal well-being. Increased fetal DNA concentrations have so far been associated with a number of pregnancy-related complications, and serum-free fetal DNA elevation has been detected in conditions including pre-eclampsia, preterm labor, invasive placenta, hyperemesis gravidarum, fetal growth restriction, fetomaternal hemorrhage, polyhydramnios, and single gene disorders such as achondroplasia, myotonic dystrophy, congenital adrenal hyperplasia, beta thalassemia, cystic fibrosis, and Huntington disease[7–19]. In the present study, we studied cfDNA levels in patients with GTD in order to test the hypothesis that cfDNA circulating in maternal plasma could provide the early detection of GTD.

Materials and methods

Sample Collection

This study included 32 patients with GTD who were referred to Gaziantep University Faculty of Medicine, Department of Obstetrics and Gynecology, and 30 patients with first-trimester pregnancies with no medical problems. This study was reviewed by the appropriate ethics committee Gaziantep University (approval number: 05/2011-04) and was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. Preliminary diagnosis of GTD was made using ultrasound. All patients had a clinical assessment and transvaginal pelvic ultrasound performed by gynecologic sonographers working in the Early Pregnancy Unit (Toshiba XARIO-XG, Toshiba) of the hospital. If the uterus was enlarged, this was supplemented by a transabdominal approach. The ultrasound criteria for suspecting molar pregnancy were cystic changes, irregularity or increased echogenicity in the decidua, chorionic tissue or myometrium. The ultrasound criteria for suspecting malignant GTD were a hypoechoic or heterogeneous, predominantly solid tumor within the uterine cavity in the presence of a positive pregnancy test. In patients with this preliminary diagnosis, the uterine cavity was evacuated through revision dilatation and curettage. The evacuated material was sent for pathology analysis. Patients with histopathologically diagnosed GTD were identified using electronic patient records and Gaziantep University Faculty Medicine Hospital Trophoblastic disease records. The control group included first-trimester pregnancies with normal biochemistry and complete blood results without medical problems. Sociodemographic, reproductive, medical and laboratory data, fetal ultrasonographic information, and patient follow-up forms were collected from both groups. Control patients diagnosed as having GTD and any other medical problems were excluded from the study.

DNA Extraction

Blood samples were collected into 12 mL EDTA collecting tubes from case patients and controls before evacuation or invasive procedures. The samples were sent to the laboratory within 15 minutes and centrifuged at 2480 rpm for 10 minutes and at 3600 rpm for 20 minutes. They were then kept in Eppendorf tubes at -80 °C. Working day materials were melted, and DNA absorbances were measured using spectrophotometry.

Polymerase Chain Reaction Analysis

DNA isolation was performed on the first serum samples taken and in accordance with the protocol of the commercial company that provided the equipment (IONTEK MagCore, RBC Bioscience). The resulting DNA material was divided into two samples: one sample was used immediately at +4 °C and the other sample was stored (for subsequent repeats) at -20 °C. The purity of the DNA obtained was determined using spectrophotometry. DNA material isolated from the plasma of the pregnant women was subject to polymerase chain reaction (PCR) analysis specific to the Y-chromosomal sequence (DYS14). Following the same protocols for the serum taken from patients, dilutions of 1/10, 1/100, and 1/1000 were prepared. Sensitivity was designated as a dilution of 1/1000. The minimum DNA (Y-chromosomal) detection limit in the study was set at 10 pg, and the X-chromosomal detection limit was set at less than 10 pg. In order to demonstrate DNA isolation efficiency, PCR analysis specific to the human glyceraldehyde-3-phosphate dehydrogenase sequence was performed. Distilled water was used as a negative control.

Statistical Analysis

Median and interquartile range are used in the presentation of numerical variables. Student's t-test and the Mann-Whitney U test were used to compare continuous variables with and without normal distribution between the groups. Any differences were considered to be significant when the p value was less than 0.05.

Outcome

This study evaluated cfDNA as the basis for the clinical outcome. The fixed variables were identified as common independent

variables (covariates) between the GTD and control groups. Then, the difference between the fixed factor of the cfDNA was investigated.

Candidate Covariates

It was crucial that the possible covariate taken as a model for GTD was clinically and biologically plausible and related to mole pregnancy in previous studies. These principles were used to determine the variables that were included in the model. Furthermore, gravida, parity, abortion, age, and weight were all identified as candidate covariates. Therefore, five candidate variables were included in the linear regression models.

Sample Size Calculation

This study used analysis of covariance (ANCOVA) because the sample size was small. In ANCOVA, there is a fixed factor and a covariate, which is used to reduce the unexplained variation in the dependent variable, cfDNA, thereby increasing its power. The dependent variable, cfDNA, was divided into two error variances: variance resulting from a covariate and unexplained error variance. If we attribute this unexplained variance to a covariate, we can reduce the unexplained error variance. Therefore, the variance in the dependent variable (i.e., the cfDNA group) is explained by the fixed factor, as well as the error variance and variance arising from the groups. In this way, the unexplained variance is reduced.

Statistical Modelling

The interaction between GTD and the control group as the fixed factor with the candidate covariates was used to form a linear regression model with a dependent variable, cfDNA. Regression modelling strategies and special transformation functions were applied to the non-standard distribution variables in the model. The residual variables were determined and ANOVA was performed after creating the linear regression models.

ANCOVA Performance and Validation

A standard calibration curve was drawn for all groups to represent the general relationship between cfDNA and the candidate covariates. This calibration curve is presented as a scatter plot in the lower panel of the correlogram matrix. The middle panel presents the distribution plots of the variables in the histogram, and the upper panels show the correlation coefficient of the variables. All statistical analyses were explained using the R (R Statistical Software, Institute for Statistics and Mathematics, Vienna, Austria) software package version 3.5.1.

Results

This study included 62 patients. Of the 32 patients diagnosed as having GTD, 16 were reported as complete moles and 16 were reported as partial moles. The control group consisted of 30 pregnant women who were healthy during the 12th

Table 1. General characteristics used to compare the control and gestational trophoblastic disease groups

	Control group n=30	GTD group n=32	p value	ANCOVA p value
Age, years [median (IQR)]	**33 (28-37.7)**	**29.50 (24-35.2)**	**0.50**	**0.009***
Gravida [median (IQR)]	2 (2-4.75)	3 (2-4)	0.32	0.04*
Parity [median (IQR)]	1 (1-2)	1 (0.7-3)	0.65	0.04*
Abortion [median (IQR)]	0 (0-1.75	0 (0-1)	0.61	0.02*
cfDNA, pg [(median (IQR)]	308 (1.25-467.2)	854.5 (2-1004)	0.02*	
Weight, kg [median (IQR)]	65.5 (60-70.7)	63.5 (59.5-73)	0.93	0.02*

Values are presented as n, median IQR: Interquartile, ANCOVA: Analysis of covariance, GTD: Gestational trophoblastic disease
*Bold values are p<0.05

Table 2. Statistical parameters used to compare the complete and partial mole groups

	Partial mole n=16	Complete mole n=16	p value
Maternal age, years [median (IQR)]	27.5 (23.7-32)	33 (24.7-43.2)	0.10
b-hCG, mIU/mL [median (IQR)]	6430 (3072-22169)	100.000 (69.820-111.677)	<0.001*
Gravida [median (IQR)]	3 (2-4)	3.5 (2-7.5)	0.40
Parity [median (IQR)]	1 (0-2)	1.5 (1-3.25)	0.30
Abortus [median (IQR)]	1 (0-1.25)	0 (0-1)	0.19
cfDNA, pg, [median (IQR)]	873.5 (32.5-1008)	764 (1-981)	0.71
Weight, kg, [median (IQR)]	61 (55.7-65.7)	69.5 (62.7-76)	0.09

*Bold values are p<0.001, IQR: Interquartile *b*-hCG: Human chorionic gonadotropin

gestational week. Tables 1 and 2 summarize the baseline clinical characteristics of the study groups. A statistically significant difference was observed between the GTD and control groups in terms of cfDNA (p=0.02) (Table 1), but there was no statistically significant difference between the complete and partial mole groups (p=0.71) (Table 2). Figures 1, 2, and 3 compare the cfDNA parameters for the groups. In terms of cfDNA as dependent variable made ANCOVA. Moreover, the participating candidate covariates showed a significant difference when compared with the fixed factor (Table 1). The performance and validation of the variables using ANCOVA are presented in the correlogram matrix (Figure 4).

Discussion

This study measured the increase in cfDNA levels in GTD in order to test the hypothesis that cfDNA circulating in maternal plasma could provide early detection of GTD. Non-parametric ANCOVA in terms of cfDNA in GTD was performed, thereby increasing its power and revealing a significant difference compared with the control group. Tjoa et al.[20] found an increase in free fetal-placental DNA in maternal serum due to trophoblastic oxidative stress, resulting in trophoblastic degeneration. Therefore, concentrations of cfDNA in maternal serum and plasma may act as a biomarker of trophoblast well-being during pregnancy, and could provide a scientific rationale for the administration of antioxidant vitamins in high-risk pregnancies. Alberry et al.[21] showed that cfDNA was the primary source of maternal plasma, which supports the hypothesis that trophoblasts are a source of cfDNA in

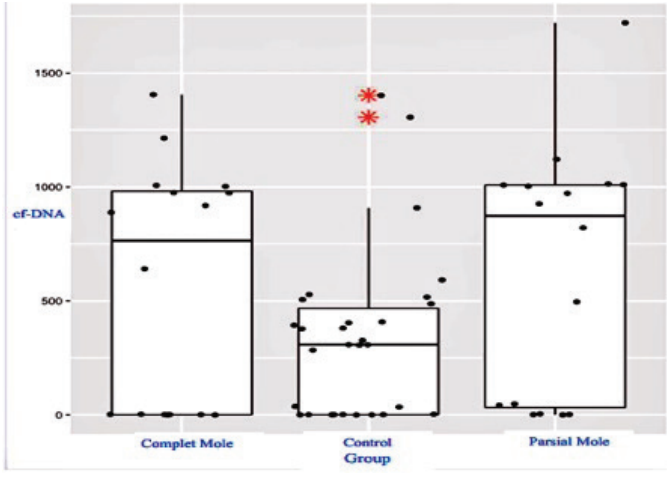

Figure 1. Plot of complete and partial mole groups according to a comparison of the cfDNA and control groups

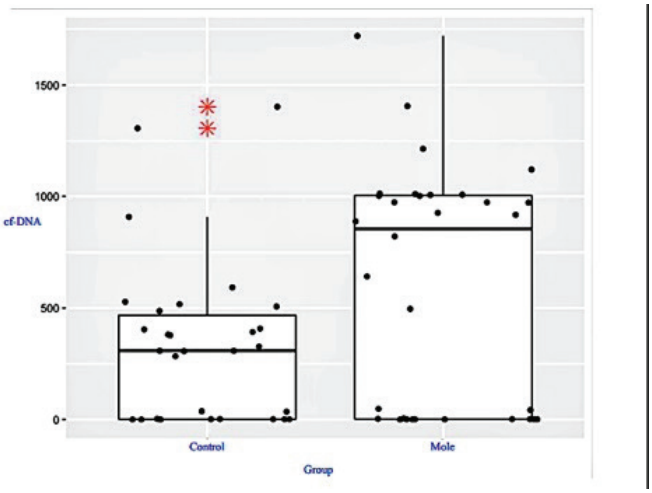

Figure 2. Plot of the GTD group according to a comparison of the cfDNA and control groups

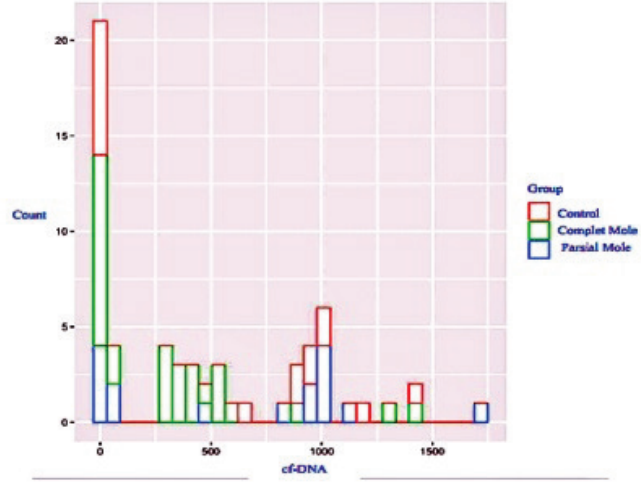

Figure 3. Plot of complete and partial mole groups according to a comparison of the cfDNA and control groups

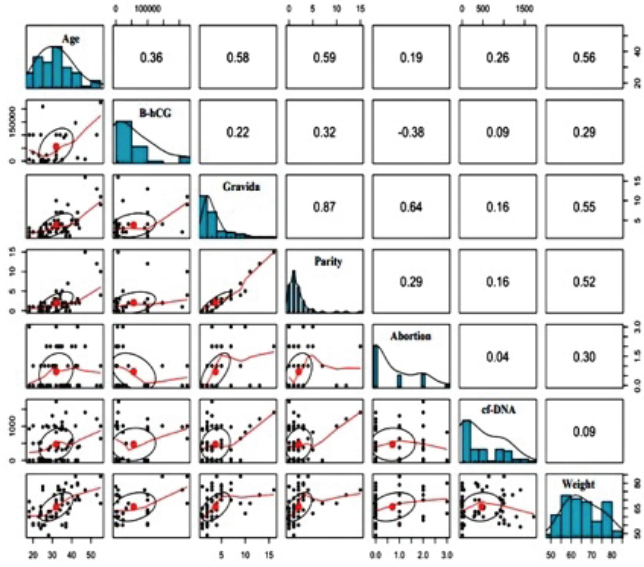

Figure 4. The lower panel of the matrix shows a scatter plot of all variables and provides the homogeneity of the calibration curves. The middle panel of the matrix shows the histogram distribution plots of the variables, and the upper panel shows the correlation coefficient

maternal plasma in anembryonic pregnancies. These two studies indicated that trophoblastic injury might cause the level of cfDNA to increase in maternal serum, and our study showed high levels of cfDNA in GTD.

Sifakis et al.[22] found that the concentration of maternal cfDNA increased at 11-13 weeks of gestation in pregnancies that experienced early-onset preeclampsia. This provides further support for the presence of impaired placentation in the pathogenesis of the disease. Yin et al.[23] found that concentrations of maternal plasma fetal DNA and total DNA increased throughout the first trimester. Significantly high levels of fetal and total DNA were also found in pregnancies that miscarried. The integration of non-invasive prenatal diagnosis into clinical care has identified new aspects of perinatal biology, mainly cfDNA, which is a new biomarker that can provide information about the placenta and can potentially be used to predict clinical problems[24]. Openshaw et al.[25] showed that cfDNA could be detected in the plasma of women with trophoblastic tumors and facilitate diagnosis. Although cfDNA could be used as a marker for identifying subjects at increased risk of developing pre-eclampsia, it is not suitable for measuring cfDNA levels to diagnose ectopic pregnancies in the early period[26,27]. The present study shows that the cfDNA increase is significant during the early period of GTD. One of the limitations of our study is that the cfDNA-DYS14 assay method was used to isolate cfDNA from the mother's plasma. This is the oldest method and does not work with new methods of study. We used this method because it was the most cost-effective given that the authors themselves met the cost of financing the tests.

Conclusion

All aspects of the pathophysiology of GTD have not yet been elucidated. Therefore, it can still lead to morbidity and mortality. In order to reduce these risks, it is necessary to focus on practical and cost-effective solutions, especially ones that are likely to assist with early diagnosis. Few studies have investigated the relationship between GTD and cfDNA; however, this is likely to change in the coming years as cfDNA becomes recognized as an essential focus of research. Based on existing studies, we can predict that maternal cfDNA levels increase due to placental damage in pathologic pregnancies. This study measured cfDNA using PCR analysis of maternal serum, and the results show significant differences between the patient and control groups. We also suggest that monitoring the maternal peripheral bloodstream for cfDNA could be significant for the early diagnosis of GTD.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of the Gaziantep University (approval number: 05/2011-04).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

Author Contributions

Concept: F.B.C., Data Collection or Processing: M.H.B., Ö.B., S.C., Analysis or Interpretation: H.Ç.Ö., Literature Search: S.S., Writing: M.H.B., M.O.

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Effect of using magnifying loupe glasses on lymphocele formation and surgical outcomes in gynecologic oncology

Jinekolojik onkolojide büyüteçli gözlük kullanımının lenfosel oluşumu ve cerrahi sonuçları üzerine etkisi

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Abstract

Objective: To investigate the effect of using magnifying loupes during surgery on surgical outcomes and lymphocele formation.

Materials and Methods: We prospectively enrolled 36 patients with gynecologic cancer who underwent pelvic and para-aortic lymphadenectomy. Age, body mass index, menopausal status, type of cancer, comorbid diseases, preoperative albumin and albumin replacement therapy, performance status, serum CA125, hemoglobin, platelets and white blood cells, surgical procedure, blood loss, blood transfusion, the count of removed lymph nodes, presence of metastatic lymph nodes, total amount of drainage, postoperative complications, operation length, and count of used hemoclips were recorded. Patients were randomized into two groups: group 1 operated using loupe glasses, and group 2, without loupes.

Results: In the loupe-negative group, total drainage volume was 6698 mL, whereas in the loupe-positive group, it was only 1049 mL ($p<0.01$). Postoperatively, the mean drainage duration was 10.6±5.1 days in loupe-negative group and 4.8±2.4 days in the loupe-positive group ($p=0.0001$). There were no differences between the two groups in terms of surgical site infections, fascial defects, and pulmonary thromboembolism ($p=0.39$, 0.33, 0.59, respectively). There was no significant difference in the number of harvested lymph nodes in patients who underwent surgery with or without loupes being used. The count of used hemoclips were 50.22±8.05 and 41.38±9.7 for the loupe-negative and positive groups, respectively ($p<0.01$). There was no lymphocele in the loupe-positive group, but we detected 5 (27.8%) lymphocele in the loupe-negative group ($p=0.05$).

Conclusion: Gynecologic oncologic surgeons can add magnifying loupe glasses to their armament and benefit from this technical device; lymphocele development, total drainage volume, length of drainage time, and clip counts can be decreased by using loupe glasses in gynecologic cancer surgery.

Keywords: Gynecologic oncology, magnifying loupe glasses, lymphocele

Öz

Amaç: Bu çalışmada cerrahide büyüteçli gözlük kullanımının cerrahi sonuçlar ve lenfosel oluşumu üzerindeki etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya pelvik ve para-aortik lenf nodu disseksiyonu yapılan 36 jinekolojik kanser hastası prospektif olarak dahil edildi. Yaş, vücut kitle indeksi, menopozal durum, kanser tipi, ek hastalıkların varlığı, operasyon öncesi albumin düzeyi ve albümin replasmanı, performans durumu, serumda CA125, hemoglobin, platelet ve beyaz küre sayısı, cerrahi prosedür, kan kaybı miktarı, kan transfüzyonu ihtiyacı, çıkarılan lenf nodu sayısı, metastatik lenf nodu varlığı, toplam drenaj miktarı, operasyon sonrası komplikasyonlar, operasyon süresi ve kullanılan hemoklip sayısı kaydedildi. Hastalar cerrahi sırasında büyüteçli gözlük kullanılan ve kullanılmayan olarak iki grupta randomize edildi.

Bulgular: Büyüteçli gözlük kullanılmayan grupta, toplam drenden gelen miktar 6698 mL iken büyüteçli gözlük kullanılan grupta sadece 1049 mL olarak tespit edildi ($p<0,01$). Cerrahi sonrası drenin kalış süresi büyüteçli gözlük kullanılmayan grupta 10,6±5,1 gün, büyüteçli gözlük kullanılan grupta 4,8±2,4 gün olarak bulundu ($p=0,0001$). İki grup arasında cerrahi alan enfeksiyonu, fasya defekti ve pulmoner emboli açısından anlamlı fark bulunmadı (sırasıyla: $p=0,39$, 0,33, 0,59). Büyüteçli gözlük kullanılan ve kullanılmayan grupta çıkarılan lenf nodu sayısında da anlamlı fark bulunmadı. Kullanılan hemoklip sayısı büyüteçli gözlük kullanılmayan ve kullanılanlarda sırasıyla 50,22±8,05 and 41,38±9,7 olarak saptandı ($p<0,01$). Büyüteçli gözlük kullanılan grupta lefosel oluşumu görülmezken, kullanılmayan grupta 5 (%27,8) hastada lenfosel izlendi.

Sonuç: Jinekolojik onkoloji cerrahları büyüteçli gözlük kullanımından fayda görebilir dolayısıyla ekipmanlarına bu gözlükleri ekleyebilirler. Jinekolojik kanser cerrahisinde büyüteçli gözlük kullanımı ile lenfosel gelişimi, toplam drenden gelen miktar, drenaj süresi ve kullanılan hemoklip sayısı azalabilir.

Anahtar Kelimeler: Jinekolojik onkoloji, büyüteçli cerrahi gözlük, lenfosel

PRECIS: In gynecologic cancer surgeries, using loupe glasses is beneficial in decreasing lymphocele formation, amount of lymphatic drainage, and hemoclip use.

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Introduction

Pelvic and para-aortic lymphadenectomy is a common procedure in gynecologic oncologic surgery to determine lymph node status and disease stage. Hemorrhage, hematoma, nerve and ureteral injury, postoperative ileus and lymphocele formation due to disruption of lymphatic drainage are well-known complications of lymphadenectomy. Usually, lymphoceles appear within 2 months after surgery and they are mostly asymptomatic. However, they may rarely affect the ureter and bladder (hydronephrosis, urinary frequency), bowel (ileus, tenesmus), or vessels (thrombosis) and cause abdominal pain. Lymphoceles may also become infected[1,2]. The incidence of lymphoceles after gynecologic cancer surgery is reported as 1-58%. There are many factors that affect the formation of lymphoceles, the count of removed lymph nodes, the extent of lymphadenectomy, status of ligated lymph vessels, use of retroperitoneal suction drainage, type of cancer, and administration of heparin for thromboembolic prophylaxis[2,3]. The success of any techniques has not been proven in the literature and there are a few reports that found a significant reduction in postoperative lymphocele formation after lymphadenectomy[4]. Loupes allow to see 2-4 times more magnification than eyes. Loupes have many benefits: wider and deeper field of view, cost, portability. Loupes are used in plastic, maxillofacial, otorhinolaryngologic, ophthalmic, cardiothoracic, and pediatric surgery[5]. The use of loupes in gynecologic oncology has been described in nerve-sparing radical hysterectomy[6].

The aim of this study was to investigate the effect of using magnifying loupes during surgery on surgical outcomes and lymphocele formation.

Materials and Methods

We prospectively enrolled 36 patients with gynecologic cancer who underwent pelvic and para-aortic lymphadenectomy between February 2016 and July 2017 at the Department of Gynecologic Oncology of Süleyman Demirel University. Age, body mass index (BMI), menopausal status, type of cancer, comorbid diseases, preoperative albumin and albumin replacement therapy, performance status [Eastern Co-operative Oncology Group (ECOG) and Karnosky], serum carcinoma antigen 125 (CA125), serum hemoglobin, platelets and white blood cells (WBC), surgical procedure, blood loss, blood transfusion, the count of removed lymph nodes, presence of metastatic lymph nodes, total amount of drainage, postoperative complications, the length of total surgical time, and count of hemoclips used were recorded.

Ethics Committee Approval: The study was approved by Local Ethics Committee of Süleyman Demirel University (approval number: 78, date: 20,07, 2016).

Statistical Analysis

Statistical analysis was performed using MedCalc. The significance level was set as $p<0.05$. Descriptive analysis was performed using the independent t-test, Mann-Whitney U, and chi-square test. These parameters were evaluated via correlation tests, and multiple regression analysis was performed for efficient parameters. Prediction of the results was evaluated using receiver operating characteristic curve analysis. Power analysis was calculated with 80% power, 5% alpha to a 50% decrease in the total amount of drainage. Patients were randomized into two groups; group 1 operated with the use of loupes and group 2, without loupes.

Results

Eighteen of 36 patients underwent surgery for endometrial cancer. Fifteen of 36 patients and 3 of 36 patients underwent surgery for ovarian cancer and cervical cancer, respectively. The demographic and surgical data of patients who underwent surgery with and without loupe magnification being used were compared. There were no statistically significant differences in age, BMI, menopausal status, and types of cancer. Additionally, we found no statistically significant difference in laboratory findings (preoperative albumin, preoperative hemoglobin, WBC, platelet counts, and CA125 levels) between the two groups (Table 1). The findings showed no statistically significant differences in surgical procedures (simple hysterectomy, radical hysterectomy, appendectomy, peritonectomy, panniculectomy, urinary and gastrointestinal system surgery), blood transfusions, fresh frozen plasma transfusions, and intraoperative blood loss (Table 2).

However, surgery with loupes decreased the count of hemoclips required to obliterate vessels. The counts of used hemoclips were 50.22±8.05 and 41.38±9.7 for the loupe-negative and positive groups, respectively ($p=0.006$). Furthermore, the use of loupes decreased the duration of surgery, but this finding was not statistically significant (5.3±1.4 h in the loupe-negative group, and 4.6±1.05 h in the loupe-positive group).

There was no significant difference in the number of harvested lymph nodes in patients who underwent surgery with or without loupes being used. The mean count of dissected pelvic lymph nodes was 20.0 in the loupe-positive group, and 26.5 in the loupe-negative group ($p=0.05$). The mean count of dissected para-aortic lymph nodes in the loupe-positive group (n=13.5) was more than in the loupe-negative group (n=12) ($p=0.93$).

Although the amount of drainage after the first and 6 hours of surgery were similar, there was a statistically significant difference after the first day, second day, and total drainage volume. In the loupe-negative group, the mean drainage volume was 454 mL for the postoperative first day, 661 mL for postoperative second day, and 6698 mL for total drainage volume, whereas in the loupe-positive group was 245 mL, 229 mL, and 1049 mL, respectively. Postoperatively, the mean duration of drainage was 10.6±5.1 days in loupe-negative group and 4.8±2.4 days in loupe-positive group ($p=0.0001$). There were no differences between the two groups in terms of surgical site infection, fascial defect, and pulmonary

thromboembolism (p=0.39, 0.33, and 0.59, respectively). Lymphocele formation was lesser in the loupe-positive group than in the other group; there was no lymphocele in the loupe-positive group, but we detected 5 (27.8%) lymphoceles in the loupe-negative group (p=0.05). Multiple regression analysis revealed a significant relationship between total drainage volume, use of loupes, BMI, hemoglobin level, count of dissected pelvic lymph nodes, metastatic pelvic lymph nodes, metastatic para-aortic lymph nodes, surgical time, type of cancer, and ECOG score (Table 3).

Table 1. Demographic results and laboratory findings of the groups

Demographic results		Surgery without loupes n=18	Surgery with loupes n=18	p value
Age		54.2±115	55.8±12.8	0.69
BMI (kg/m^2)		32.55±5.9	32.27±8.6	0.91
Menopausal status	Pre-menopause	5 (27.8%)	14 (77.7%)	0.999
	Post-menopause	13 (72.2%)	4 (22.2%)	
Cancer type	Endometrium	7 (38.9%)	11 (61.1%)	0.06
	Over	8 (44.4%)	7 (38.9%)	
	Cervix	3 (16.6%)	0 (0%)	
Preoperative albumin (g/dL)		3.34±0.52	3.42±0.49	0.65
Number of patients treated with albumin		11 (61.1%)	10 (55.6%)	0.999
Median hemoglobin levels of patients (g/L)		12.9 (9.9-15.9)	12.3 (9.9-14.3)	0.19
WBC (x10^3/µL)		8.1±3.2	10.1±4.7	0.14
PLT (x10^3/µL)		291.3±92.7	301.3±127.3	0.78
CA125 U/mL		20 (5.8-619)	38 (8.6-1990)	0.23

BMI: Body mass index, WBC: White blood cell, PLT: Platelets, CA125: Carcinoma antigen 125

Table 2. Evaluation of surgical procedures between the two groups

Parameters	Loupe negative n=18	Loupe positive n=18	p value
Prior abdominal surgery	9 (50%)	4 (22.2%)	0.16
Type1 hysterectomy	14 (77.8%)	17 (94.4%)	0.33
Type 2-3 hysterectomy	3 (16.7%)	0 (0%)	0.22
Gastrointestinal system surgery	6 (33.3%)	9 (50%)	0.49
Appendectomy	5 (27.8%)	9 (50%)	0.30
Urinary system surgery	2 (11.1%)	1 (5.6%)	0.999
Peritonectomy	2 (11.1%)	1 (5.6%)	0.999
Panniculectomy	0 (0%)	1 (5.6%)	0.999

Discussion

This is the first study to analyze the effect of wearing loupes on lymphadenectomy, lymphocele formation, and surgical outcomes in gynecologic oncology surgery. In our study, loupe use reduced surgical time but there was no statistically significance (5.3±1.4 hours in loupe-negative group, and 4.6±1.05 hours in loupe-positive group). We found that the use of loupes was an effective method to reduce the amount of drainage (6698.33±5552.22 mL in loupe-negative group, and 1049.44±943.68 mL in the loupe-positive group, (p=0.002), consequently, reducing postoperative albumin replacement in the loupe-positive group (p=02).Lymph node dissection is the most common etiology of lymphocele and lymphatic leakage[2,7]. Previous studies have reported different results about lymphocele incidence. According to these studies, in patients who undergo gynecologic cancer surgery, the incidence of lymphocele is 1-58%. This wide range may be due to different types of cancer, the presence of symptoms, differences between medical centers, differences between types of surgical techniques and intraoperative procedures, and finally different methods for diagnosis. In our study, the lymphocele rate was 27.5% in the loupe-negative group, whereas no lymphocele was found in the loupe-positive group (p=0.05). In a study by Zikan et al.[7] the highest risk of lymphocele formation was reported to be in ovarian cancer, whereas the highest rate was reported in cervical cancer by Kim et al.[2]. Another study reported no association between lymphocele formation and cancer type[8]. Our study showed no significant difference between the

Table 3. The results of multiple regression analysis

Parameters	p value	r partial	Coefficient (15147.9036)
Use of loupes	**0.0004***	-0.6519	-4614.0759
BMI	**0.0164***	0.4751	191.8746
Hemoglobin level	**0.0002***	-0.6700	-1378.9265
Number of removed pelvic lymph node	**0.0017***	0.5960	143.4657
Pelvic lymph node metastasis	**0.0001***	-0.6901	-7292.1707
Paraaortic lymph node metastasis	**0.0098***	-0.5064	-5272.1997
Duration of operation	**0.0166***	0.4742	1094.9942
Type of cancer	**0.0075***	-0.5213	-2623.5521
Age	0.1685	-0.2842	-71.7999
White blood cell	0.1422	-0.3021	-192.6155
Blood transfusion	0.2089	0.2603	812.6138
ECOG score	**0.0001***	0.7031	4167.0916

BMI: Body mass index, ECOG: Eastern Co-operative Oncology Group

lymphocele rate and type of gynecologic cancer. However, there are no studies about the relationship between the amount of lymphatic drainage and type of cancer. We found that there was more lymphatic drainage in cervical cancer ($p<0.05$). However, the number of patients included in our study is the main limitation to analyze lymphocele formation in different gynecologic cancers. Gallotta et al.[8] found that the use of laparoscopic clips reduced lymphocele rates in gynecologic cancer surgeries. Magnification with loupes may help surgeons to correctly identify lymphatics and vessels. Therefore, it may decrease the total number of clips required and decrease the total lymphatic drainage and lymphocele formation.

There are some studies about the relationship between lymphatic drainage volume and the count of dissected lymph nodes[10]. According to several studies, for endometrial and cervical cancers, and for ovarian cancers, 11 and 20 lymph nodes, respectively, were accepted as sufficient and effective in pelvic lymphadenectomy[11-13]. There are no data about the maximum count of dissected lymph nodes in gynecologic cancers. In our study, the mean count of dissected pelvic lymph nodes was 23 in endometrium cancer, and 26 and 28 in ovarian and cervical cancers, respectively. Additionally, the mean count of dissected pelvic lymph nodes was 26 in the loupe-negative and 20 in the loupe-positive groups. According to previous reports, the optimal count of para-aortic lymph nodes is 10 for gynecologic cancers. A study found a positive correlation between the count of dissected para-aortic lymph nodes and the formation of chylous ascites[14]. In our study, the mean count of dissected para-aortic lymph nodes was 12 in the loupe-negative group and 13.5 in the loupe-positive group ($p=0.93$). Nevertheless, we found no significant relationship between the count of dissected para-aortic lymph nodes and the amount of lymphatic drainage.

Study Limitations

We analyzed whether using loupes decreased the most common complications of lymph node dissection; prolonged lymphatic discharge, prolonged drainage use, and lymphocele formation and hemorrhage. More studies are needed to evaluate oncologic outcomes. The main limitation of our study was the number of patients included to evaluate lymphocele formation; however, there was an enormous decrease in total drainage volume. This large difference decreased the number of patients required to randomize for a sufficient power.

Conclusion

In gynecological cancer surgeries, using loupes can decrease lymphocele formation and the amount of lymphatic drainage. Therefore, drains can be removed earlier as a part of enhanced recovery after surgery. Moreover, magnification can be beneficial in order to correctly apply clips to target vessels and decrease the number of clips required. This may reduce complications as well as the total cost of surgery. Although loupe magnification is used in many surgical practices, it is still yet not common in gynecologic oncologic procedures. More studies should be undertaken the evaluate the results of gynecologic surgery performed with loupes.

Ethics

Ethics Committee Approval: The study was approved by the Süleyman Demirel University Local Ethics Committee (approval number: 78 date: 20.07.2016).

Informed Consent: Consent forms were filled out by patients at the hospitalization time.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.E., S.C.İ., F.A., Concept: E.E., Design: E.E., F.A., Data Collection or Processing: F.A., Analysis or Interpretation: S.C.İ., M.G., Literature Search: F.A., S.C.İ., J.R., Writing: S.C.İ., J.R., F.A.

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Impact of diabetes on gastrointestinal and urinary toxicity after radiotherapy for gynecologic malignancy

Jinekolojik tümörlerde radyoterapi sonrası gastrointestinal ve üriner toksisite üzerinde diyabetin etkisi

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Abstract

Objective: Although diabetes is a common co-morbidity in patients with gynecologic cancer, information about its impact on radiation toxicity in patients with gynecologic cancer treated with external pelvic irradiation is scarce. We aimed to investigate the relation of diabetes with acute toxicity in patients with gynecologic tumors who underwent pelvic +/- paraaortic radiotherapy.

Materials and Methods: One hundred twenty-nine patients with endometrium or cervix carcinoma were enrolled in the study. Demographic features, presence of diabetes, incidence and severity of upper gastrointestinal (UGIS), lower gastrointestinal (LGIS), and urinary symptoms were recorded from files. Correlation and logistic regression analysis was used to determine the impact of diabetes, age, chemotherapy, paraaortic irradiation on toxicities, and a prediction model was developed.

Results: The median age of 77 patients with endometrium cancer and 52 cervix cancer was 61 (range, 25-92) years, and 28 (21.7%) of them had diabetes. The median pelvic and tumor/tumor bed dose was 5040+247.65 cGy and 5040+222.91 cGy, respectively. Age and Gr 0 UGIS toxicity were significantly related (p=0.047). LGIS Gr 0 toxicity was found to be significantly higher in patients with diabetes (p=0.045). Gr 0 and 2 UGIS toxicities were both found to be significantly correlated with paraaortic irradiation (both p<0.001). Diabetes is also an important determinant on UGIS toxicity in patients who underwent paraaortic irradiation.

Conclusion: The correlation we found between toxicity and diabetes, concurrent chemotherapy or paraaortic radiation necessitates special care and risk stratification for patients with diabetes. Further prospective studies with long follow-up and larger patient groups are warranted.

Keywords: Diabetes, gynecologic tumor, radiation toxicity, pelvic radiotherapy

Öz

Amaç: Jinekolojik tümörlerde diyabet oldukça sık rastlanan bir komorbidite olmasına rağmen eksternal pelvik radyoterapi alan bu hastalarda diyabetin radyasyon toksisitesi üzerine etkisi ile ilgili bilgiler oldukça sınırlıdır. Bu çalışmada pelvik +/- paraaortik radyoterapi alan jinekolojik malignite hastalarında diyabetin akut radyasyon toksisitesi üzerine etkisinin araştırılması amaçlandı.

Gereç ve Yöntemler: Yüz yirmi dokuz endometrium veya serviks kanserli hasta çalışmaya dahil edildi. Demografik özellikler, diyabet bilgisi, üst gastrointestinal (UGIS), alt gastrointestinal (LGIS) ve üriner semptomlar, tedavi bilgileri dosyalardan elde edildi. Diyabetin ve tedavi modalitelerinin toksisite üzerine etkisi korelasyon ve lojistik regresyon analizi ile değerlendirildi. Ek olarak toksisite prediksiyon modeli geliştirildi.

Bulgular: Yetmiş yedi endometrium ve 52 serviks kanserli olgunun medyan yaşı 61 (25-92) yıl olarak bulundu ve 28 (%21,7) hastada diyabet mevcut idi. Medyan pelvik ve tümör/tümör yatağı dozu sırasıyla 5040+247,65 cGy ve 5040+222,91 cGy idi. Yaş ve Gr 0 UGIS toksisitesi anlamlı düzeyde ilişkili olarak bulundu (p=0,047). Gr 0 LGIS toksisitesi diyabetik hastalarda anlamlı düzeyde yüksek idi (p=0,045). Gr 0 ve 2 UGIS toksisitesi ile pararaortik radyoterapi arasında istatistiksel anlamlı korelasyon saptandı (both p<0,001). Paraaortik radyoterapi uygulanan hastalarda UGIS toksisitesi açısından diyabet varlığının belirleyici rol oynadığı saptandı.

Sonuç: Toksisite ile diyabet, eş zamanlı kemoterapi, paraaortik radyoterapi arasında bulduğumuz ilişki diyabetik hastaların radyoterapi öncesinde özenle değerlendirilmesi ve risklerinin belirlenmesi gerektiğini göstermektedir. Daha uzun takipli ve daha fazla hasta sayısı ile yapılacak yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Diyabet, jinekolojik tümörler, radyasyon toksisitesi, pelvik radyoterapi

PRECIS: In 129 gynecologic tumor patients we investigated the impact of diabetes on radiation toxicity.

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Introduction

Diabetes is one of the common comorbidities in patients with cancer, leading to long-term complications[1]. The impact of diabetes mellitus on radiation toxicity of lung and rectum is reported by a number of previous studies. Normal lung tissue toxicity in terms of radiation pneumonitis is proved to be higher in diabetic patients with lung cancer[2-5]. Radiographic radiation-induced lung injury has also found to be associated with the presence of diabetes after lung stereotactic body radiation therapy, most prominently early after treatment. Increased caution while treating patients with diabetes is strongly suggested[6]. In patients with prostate cancer treated with pelvic radiotherapy, the association of a high incidence and high-grade incontinence and sexual function[7], other genitourinary symptoms[8] with diabetes has also been reported. Even in patients with localized prostate cancer, a negative effect of diabetes on late gastrointestinal and urinary toxicities has been found[9]. Kalakota et al.[8] suggested taking the relationship into consideration in patients with diabetes, especially among those receiving dose-escalated RT or with a history of surgery. Even the newer techniques such as intensity modulated radiotherapy (IMRT) or image-guided radiotherapy, anatomic close proximity of rectum and lower urinary tract causes symptoms leading to impairment in quality of life[10]. The effect of diabetes on radiation toxicity has been the subject of debate in many studies with patients with prostate carcinoma. However, it has not been investigated in gynecologic tumors; therefore, we aimed to determine whether diabetes had any impact on the acute radiation adverse effects of women who underwent pelvic radiation therapy for gynecologic malignancies.

Materials and Methods

The study was approved by the Scientific Research Ethics Committee of the medical faculty of Süleyman Demirel University (protocol code: 2019/139). All procedures were performed in terms of the ethical standards of the institutional research committee in alliance with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived owing to the retrospective nature of the study.

The medical records and laboratory data of 129 patients with gynecologic tumors who underwent pelvic +/- paraaortic radiotherapy from September 2011 to January 2019 were evaluated retrospectively. The inclusion criteria were: (1) patients who were diagnosed and histologically confirmed as having endometrium or cervix carcinoma; (2) patients who underwent primary radical chemoradiotherapy or adjuvant radiotherapy; (3) patients who received a dose of radiotherapy ranging between 4500 cGy-5400 cGy in 25-30 fractions; and (4) patients who acquired 3D conformal radiotherapy (3DCRT) or IMRT. The exclusion criteria were: (1) patients with missing data in terms of toxicity recording; (2) patients who had known chronic symptomatic proctitis; (3) symptomatic hemorrhoids; (4) and those who had known previous urinary or rectal surgery.

Taking the above-mentioned criteria into consideration, 77 patients with endometrium carcinoma and 52 with cervix carcinoma were included in the study. Radiotherapy was given to the target volume delineated with the guidance of preoperative fluorodeoxyglucose-positron emission tomography/computed tomography regarding high sensitivity, specificity, and negative predictive values for detecting pelvic and paraaortic lymph node metastasis as reported previously[11].

The severity of radiation-induced upper gastrointestinal (UGIS) (nausea, vomiting, loss of appetite, weight loss), lower gastrointestinal (LGIS) (hemorrhoids, diarrhea, rectal bleeding) and urinary symptoms (dysuria, pollakuria, polyuria, hematuria, urgency) are graded and recorded according to the Radiation Oncology Toxicity Grading (RTOG) grading system (Table 1)[12]. Correlation and logistic regression analysis were used to determine the impact of diabetes status, age, concomitant chemotherapy, and paraaortic irradiation on the grades of toxicities. Additionally, a toxicity probability predictor model was developed.

Results

The median age of entire cohort was 61 (range, 25-92) years. The clinical and demographic characteristics of the study participants are shown in Table 2. Radiotherapy was given with curative intent in 23 patients who were inoperable and 6 patients with cervix carcinoma who underwent paraaortic lymphatic sampling. The number of patients treated with 3DCRT, static IMRT, and IMRT were 86, 37, and 6, respectively. The median pelvic dose was 5040 (4500-6120) cGy, total dose to tumor or tumor bed was 5040 (4500-6120) cGy. Of the 129 patients, 28 had type II diabetes.

Statistical Analysis

UGIS toxicity was significantly related with concomitant chemotherapy (Figure 1a) and paraaortic radiotherapy (Figure 1b) with $p<0.001$. The difference in UGIS toxicity according to the presence of diabetes was not significant. With Pearson correlation, age and Gr 0 UGIS toxicity was found significantly related ($p=0.047$). When paraaortic radiotherapy and UGIS toxicity correlation was evaluated using the z-score test, Gr 0 and 2 toxicities were both found to be significantly correlated (both $p<0.001$); however, the result was statistically nonsignificant for Gr 1 ($p=0.383$). Concomitant chemotherapy and UGIS toxicity was found to be statistically significantly correlated in the chi-square test ($p=0.02$). LGIS Gr 0 toxicity was found to be related with diabetes ($p=0.045$). Pearson's test revealed a significant correlation between diabetes and LGIS ($p=0.037$). In addition, a significant correlation with concurrent chemotherapy was found ($p=0.042$). No significant correlation was found with diabetes and urinary toxicity in the z test. Concurrent chemotherapy and urinary toxicity correlation was evaluated using z scores, which revealed statistical significance for Gr 2 toxicity ($p=0.012$). UGIS, LGIS, and GUS toxicity

Table 1. Radiation Oncology Toxicity Grading toxicity grade scoring system

Tissue	Grade 1	Grade 2	Grade 3	Grade 4
UGIS	- Anorexia with ≤5% weight loss from pretreatment baseline - Nausea not requiring antiemetics - Abdominal discomfort not requiring medication	- Anorexia with ≤15% weight loss from pretreatment baseline - Nausea-vomiting requiring antiemetics - Abdominal pain requiring analgesics	- Anorexia with >15% weight loss from pretreatment baseline requiring NG tube or parenteral support. - Nausea-vomiting requiring tube or parenteral support - Severe abdominal pain, despite medication - Hematemesis or melena abdominal distention (flat plate radiograph demonstrates distended bowel loops)	- Ileus, - Subacute or acute obstruction, - Perforation, - GI bleeding requiring transfusion - Abdominal pain requiring tube decompression or bowel diversion
LGIS	- Increased frequency change in quality of bowel habits not requiring medication - Rectal discomfort not requiring analgesics	- Diarrhea requiring parasympatholytic drugs (e.g. Lomotil) - Mucous discharge not necessitating sanitary pads - Rectal or abdominal pain requiring analgesics	- Diarrhea requiring parenteral support - Severe mucous or blood discharge necessitating sanitary pads - Abdominal distention (flat plate radiograph demonstrates distended bowel loops)	- Acute or subacute obstruction, fistula or perforation; - GI bleeding requiring transfusion; - Abdominal pain or tenesmus requiring tube decompression or bowel diversion
GUS	- Frequency of urination or nocturia twice pretreatment habit - Dysuria, urgency not requiring medication	- Frequency of urination or nocturia that is less frequent than every hour. - Dysuria, urgency, bladder spasm requiring local anesthetic (e.g. Pyridium)	- Frequency with urgency and nocturia hourly or more frequenty - Dysuria, pelvis pain or bladder spasm requiring regular, frequent narcotic - Gross hematuria with/without clot passage	- Hematuria requiring transfusion - Acute bladder obstruction not secondary to clot passage, ulceration, or necrosis

UGIS: Upper gastrointestinal, LGIS: Lower gastrointestinal, GI: Gastrointestinal, NG: Nasogastric

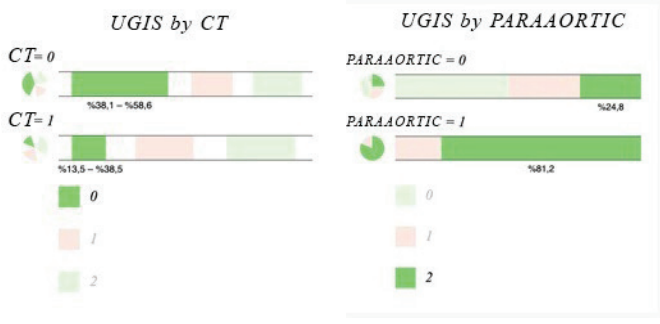

Figure 1a-b. Upper gastrointestinal toxicity according to grades with concomitant chemotherapy (a) and paraaortic radiotherapy (b)
UGIS: Upper gastrointestinal

ratios according to diabetes and correlation results are given in Table 3. The correlation results of chemotherapy and paraaortic radiotherapy with toxicity in relation with having diabetes are summarized in Table 4. An ordinal regression analysis was performed and variables with p<0.1, which were concurrent chemotherapy, paraaortic irradiation, diabetes, blood glucose level, and age, were included in the model. A toxicity prediction model for pelvic radiation treatment in this study was developed using a regression test. Paraaortic irradiation was significant in predicting LGIS (p=0.09) and UGIS toxicity (p<0.001). The standard error and confidence intervals are shown in Figure 2a and 2b. The prediction values are shown in Table 5. Diabetes was not found to be predictive for UGIS toxicity. No significant predictor was found for urinary toxicity in the regression analysis.

Discussion

In this study, we evaluated the impact of diabetes on the incidence and severity of UGIS, LGIS, and urinary acute radiation toxicity in patients with gynecologic cancer who underwent pelvic +/- paraaortic radiotherapy. The relation of diabetes with radiation toxicity should not be unexpected if one considers previous reports indicating increased radiation-induced pulmonary toxicity after thoracic irradiation and proctitis or urinary incontinence consequent to pelvic radiotherapy with diabetes mellitus[2-5,7]. This connection has been enucleated with a number of theories. The increased morbidity and mortality rates in patients with diabetic cancer after surgery was reported previously and attributed to reduced leukocyte activities, such as phagocytosis and opsonization, which consequently intervene in natural host immunity[13,14]. Radiation causes more significant damage in fast proliferating tissues such as the lining epithelium of the skin, gastrointestinal tract, and blood vessels. The vascular configuration, which plays the major role in the repair of radiation damage, becomes disrupted through an activated coagulation system, decreased blood flow, thrombosis, and capillary necrosis[14-16]. Diabetes leads to an impairment in the function of vessel endothelial lining and dilatation of microvasculature[14], resulting in dysfunctional tissue repair. Our results revealed that patients without diabetes

mellitus had a significantly lower incidence of LGIS toxicity in patients with diabetes, which we proved with a significantly higher ratio of Gr 0 toxicity. This is incompatible with the results of Alashkham et al.[17], who reported significantly higher rates (p<0.001) of high-grade proctitis in diabetic prostate patients after pelvic radiotherapy and suggested diabetes as a significant predictor of proctitis after pelvic radiotherapy. Another study by Zelefsky et al.[9] also reported a significant association between late gastrointestinal and urinary toxicities with diabetes in patients with localized prostate cancer treated with 3DCRT, in conformity with our findings. The results of our data analysis revealed that the incidence of proctitis was significantly lower in patients who were not diabetic. A study on the impact of diabetes on radiation toxicity suggested that diabetic status increased the risk of radiation toxicity and reset the onset of symptoms to an earlier time and slowed down its resolution[17]. The lower incidence of LGIS in patients with diabetes may be attributed to the scant number of diabetic cases and variable extrinsic factors influencing acute toxicity symptoms if one takes into consideration that previous studies suggested on late toxicities. Radiation dose and technique such as 3DCRT or IMRT

Table 2. Clinical and demographic characteristics of the patients

		Overall
Age	Median	61
	Min	25
	Max	92
Primary	Endometrium	77
	Cervix	52
Paraaortic RT	Yes	16
	No	113
Paraaortic RT dose	Median	4500 cGy
	Min	4500 cGy
	Max	4680 cGy
Pelvic RT dose	Median	5040 cGy
	Min	4500 cGy
	Max	6000 cGy
Boost RT dose	Median	540 cGy
	Min	180 cGy
	Max	1080 cGy
RT technique	3DCRT	86
	Static IMRT	37
	IMRT	6
Chemotherapy	Yes	42
	No	87

RT: Radiotherapy, IMRT: Intensity modulated radiotherapy, 3DCRT: 3D conformal radiotherapy

Table 3. Lower gastrointestinal, upper gastrointestinal and GUS toxicity with diabetes and p values

	Toxicity	Dm (-) (%)	Dm (+) (%)	P value
LGIS	Gr 0	30 (29.7)	14 (50)	0.045
	Gr 1	31 (30.7)	5 (17.9)	0.18
	Gr 2	39 (38.6)	9 (32.1)	0.53
	Gr 3	1 (0.99)	0 (0)	0.59
UGIS	Gr 0	42 (41.6)	10 (35.7)	0.575
	Gr 1	29 (28.7)	7 (25)	0.698
	Gr 2	30 (29.7)	11 (39.3)	0.335
GUS	Gr 0	68 (67.3)	20 (71.4)	0.680
	Gr 1	19 (18.8)	5 (17.9)	0.909
	Gr 2	14 (13.9)	3 (10.7)	0.663

LGIS: Lower gastrointestinal, UGIS: Upper gastrointestinal

Table 4. Upper gastrointestinal, lower gastrointestinal and urinary toxicity ratios in diabetic and non-diabetic patients according to chemotherapy and paraaortic irradiation

	DM		LGIS				UGIS			Urinary		
			0	1	2	3	0	1	2	0	1	2
CT (-)	0	65	29.2%	29.2%	40%	1.5%	50.7%	24.6%	24.6%	64.6%	15.4%	20%
	1	22	54.5%	18.2%	27.3%	0%	40.9%	22.7%	36.4%	63.6%	22.7%	13.6%
CT (+)	0	36	30.6%	33.3%	36.1%	0%	25%	36.1%	38.9%	72.2%	25%	2.7%
	1	6	33.3%	16.7%	50%	0%	16.7%	33.3%	50%	100%	0%	0%
PA RT (-)	0	91	28.6%	28.6%	41.7%	1.1%	46.2%	29.7%	24.2%	65.9%	18.7%	15.4%
	1	22	50%	18.2%	31.8%	0%	45.5%	27.3%	27.3%	63.6%	22.7%	13.6%
PA RT (+)	0	10	40%	50%	10%	0%	0%	20%	80%	80%	20%	0%
	1	6	50%	16.7%	33.3%	0%	0%	16.7%	83.3%	100%	0%	0%

DM: Diabetes mellitus, 0: Diabetes negative, 1: Diabetes positive, CT: Chemotherapy, PA RT: Paraaortic radiotherapy, LGIS: Lower gastrointestinal, UGIS: Upper gastrointestinal

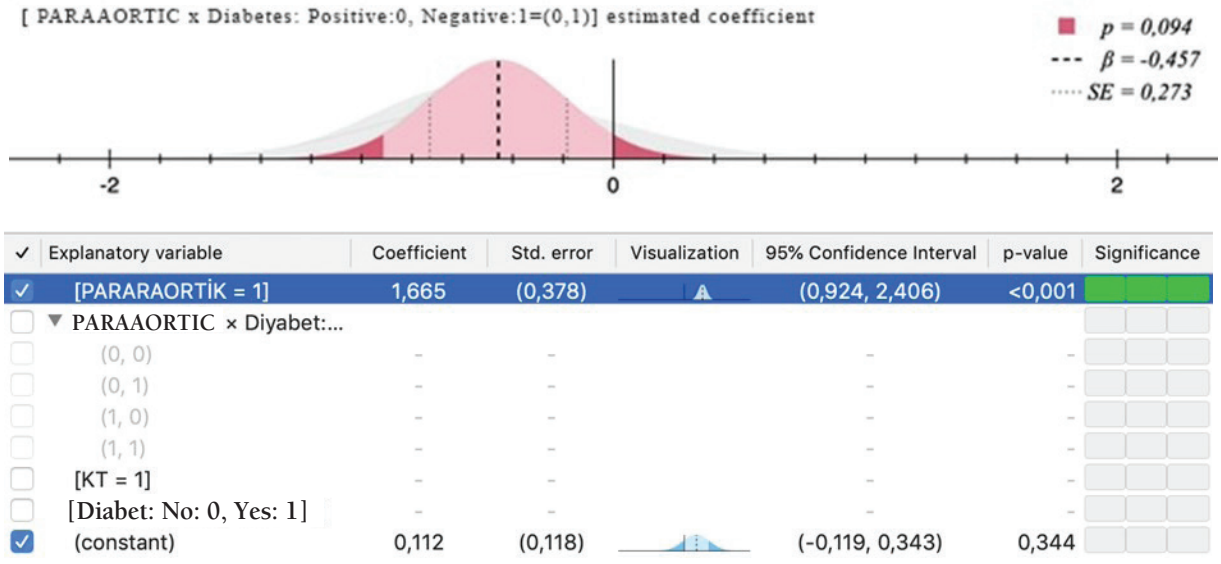

Figure 2 a-b. Standard error and confidence intervals for predictor effect of pararaortic irradiation

may affect radiation toxicity. However, dose was not found to be significant in our study. We were unable to investigate differences between radiation toxicity in insulin or medication using patients with diabetes because all of our patients used oral medications. This issue was mentioned in the study by Kalakota et al.[8] and the authors reported no difference in the incidence of gastrointestinal and urinary toxicity between insulin or oral medications. To the best of our knowledge, no evidence has been presented in terms of the association between LGIS toxicity and age[18]; however, we found a significant correlation between age and Gr 0 UGIS toxicity using Pearson's correlation analysis (p=0.047). A major issue of published literature is the lack of any uniform and reliable method to evaluate and record proctitis, a number of studies did not even record the severity as grades[19-24]. In this study, we used the RTOG grading system to evaluate the severity of radiation-induced proctitis[25,26] as a reliable and well-recognized method. All our patients were treated with a median pelvic dose of 5040 (range, 4500-6120) cGy, the total dose to tumor or tumor bed was 5040 (range, 4500-6120) cGy in a median 28 (range, 25-32) days; therefore the data were inadequate for evaluating toxicity in patients with diabetes according to higher or lower radiation doses.

One of the limitations of our study is the limited number of patients, which led to inadequate data to enable comment, especially due to the small group with diabetes. The correlations we found and prediction values may be discordant, which can be attributed to the limited number of cases. The second important limitation is the retrospective design, which causes doubts in the uniformity of toxicity data. However, this can be used as a model for pilot studies because this paper is the first to investigate the impact of diabetes on radiation toxicity in women who underwent pelvic +/- paraaortic radiotherapy for gynecologic cancer.

Table 5. Predicted UGIS and LGIS toxicities with diabetes and paraaortic irradiation

			Paraaortic RT (+)	Paraaortic RT (-)
Predicted UGIS toxicity	DM (+)	Gr 0	3.8%	45.6%
		Gr 1	12.9%	30.2%
		Gr 2	83.3%	24.3%
Predicted LGIS toxicity	DM (+)	Gr 0	45.8%	45%
		Gr 1	27.7%	27.8%
		Gr 2	26.2%	26.9%
		Gr 3	0.3%	0.3%
	DM (-)	Gr 0	50.7%	28.7%
		Gr 1	26.7%	28.1%
		Gr 2	22.5%	42.2%
		Gr 3	0.2%	1%

LGIS: Lower gastrointestinal, UGIS: Upper gastrointestinal, DM: Diabetes mellitus, RT: Radiotherapy

Conclusion

Our results revealed a significantly lower incidence of LGIS toxicity in nondiabetic patients.

Taking the correlation we found between toxicity and concurrent chemotherapy or paraaortic radiation into consideration, special care should be given to patients with diabetes and risk stratification before radiotherapy. Further prospective studies are recommended to evaluate late toxicity in patients with diabetes, taking the longevity of diabetes history into consideration, and to investigate whether it is controlled using specific laboratory data such as glycated hemoglobin.

Ethics

Ethics Committee Approval: The study was approved by the Scientific Research Ethics Committee of Medical Faculty of Süleyman Demirel University (protocol code: 2019/139).
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.E., Concept: E.E., Design: E.Ö., Data Collection or Processing: J.R., Analysis or Interpretation: E.E., Literature Search: E.Ö., Writing: E.Ö.
Conflict of Interest: No conflict of interest was declared by the authors.
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Bone recurrence after radical hysterectomy and lymphadenectomy in early-stage cervical cancer

Erken dönem servikal kanserde radikal histerektomi ve lenfadenektomi sonrası kemik nüksü

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Abstract

Objective: To present the clinical, surgical, and pathologic features of bone recurrence in patients who underwent radical hysterectomy for early-stage uterine cervical cancer.

Materials and Methods: Data of 412 patients who underwent type III radical hysterectomy and pelvic ± paraaortic lymphadenectomy for stage 1B-2A epithelial cervical cancer were reviewed. Seven (1.7%) patients with bone recurrence in the first recurrence were included in the study.

Results: The median follow-up of the main cohort (n=412) was 46 (range=1-300) months. In this period, recurrence developed in 53 (12.9%) patients and recurrence was observed in bone in 13.2% (7 of 53) of these recurrences. Time to recurrence ranged from 9 to 45 months. Of the recurrences, five were in the axial skeleton and two were in the appendicular skeleton. Recurrence was observed in lumbar vertebrae in three patients, thoracic vertebrae in one patient, sacral vertebrae in one patient, lumbosacral vertebrae in one patient, and the left femur in two patients. Four patients had multiple recurrence in 3 patients despite isolated bone recurrence. Patients with multiple recurrences died within 6-25 months. All isolated bone recurrences were in the axial skeleton. Complete clinical response with salvage therapy was achieved in two patients with isolated bone recurrence.

Conclusion: Complete clinical response and long postoperative survival can be achieved with salvage treatment when bone recurrence is solitary in cervical cancers.

Keywords: Bone recurrence, uterine cervical cancer, survival, salvage therapy

Öz

Amaç: Erken evre uterin servikal kanser için radikal histerektomi yapılan hastalarda kemik nüksünün klinik, cerrahi ve patolojik özelliklerini sunmayı amaçladık.

Gereç ve Yöntemler: Evre 1B-2A epitelyal servikal kanser için tip 3 radikal histerektomi ve pelvik ± paraaortik lenfadenektomi uygulanan 412 hastanın verileri gözden geçirildi. Çalışmaya ilk nüksde kemik nüksü olan 7 hasta (%1,7) dahil edildi.

Bulgular: Ana kohortun ortanca takip süresi (n=412) 46 aydı (1-300 ay). Bu dönemde, 53 hastada (%12,9) nüks gelişti ve bu nükslerin %13,2'sinde (53) 7'sinde kemikte nüks gözlendi. Nüks zamanı 9 ila 45 ay arasında değişmekteydi. Nükslerin 5'i aksiyal iskelet, 2'si apendiküler iskeletteydı. Üç hastada lomber vertebra, 1 hastada torasik vertebra, 1 hastada sakral vertebra, 1 hastada lumbosakral omur ve 2 hastada sol femur saptandı. Dört hastada izole kemik nüksüne rağmen 3 hastada çoklu nüks vardı. Çoklu nüks olan hastalar 6-25 ay içerisinde öldüler. İzole kemik nükslerinin tümü aksiyal iskelette idi. İzole kemik nüksü olan 2 hastada kurtarma tedavisi ile tam klinik yanıt sağlandı.

Sonuç: Servikal kanserlerde kemik nüksü tek olduğunda, salvage tedavisi ile tam klinik yanıt ve postoperatif uzun sağkalım sağlanabilir.

Anahtar Kelimeler: Kemik nüksü, rahim rahim ağzı kanseri, sağkalım, kurtarma tedavisi

Introduction

Uterine cervical cancer (CC) is the third most common cause of cancer having the highest mortality rate in the female reproductive system[1]. Prognostic factors of CC are based on stage, patient age, type and size of tumor, lymph node metastases, parametrial invasion, and lymphovascular space invasion[2,3]. Mostly, recurrence occurs within 2 years after primary treatment and 90% of patients with recurrence die[4,5]. The 10-year recurrence rate is reported as 3% for stage IA, 16%

PRECIS: To identify the possible risk factors for postpartum urinary retention.

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for stage IB, 31% for stage IIA, 26% for stage IIB, 39% for stage III, and 75% for stage IVA[6].

Just like other solid tumors, CC spreads through direct invasion, and lymphatic and hematogenous dissemination. Distant metastasis to other organs such as lung, bone, braini and liver uses the hematogenous route primarily[7,8]. Distant organ metastasis is most commonly seen in lungs (21%), bone (16%), para aortic nodes (11%), the intestinal space (8%), and supraclavicular lymph nodes (7%). The number and site of metastasis are important for survival. The median survival is 24 weeks in lymphatic recurrence, whereas it is only 12 weeks in metastasis to other organs[6]. Patients with stage I-IIA CC who undergo surgery have bone recurrence in the first 2 years of the postoperative period, and usually the recurrence occurs in axial bone, especially in the vertebra[9,10]. The median survival after bone recurrence is reported as between 5 and 12 months[9-12]. In this research, our aim was to evaluate the clinical, surgical, and pathologic factors in patients with bone recurrence after type III radical hysterectomy with pelvic ± para aortic lymphadenectomy for CC.

Materials and Methods

Between 1993 and 2018, 412 patients with stage IB-IIA epithelial CC as classified by the International Federation of Obstetricians and Gynecologists (2014) were treated with laparotomy and type III radical hysterectomy with pelvic ± paraaortic lymphadenectomy, and their data were reviewed. Seven (1.7%) patients who had the first recurrence in bone were included. Data of the patients' clinical findings, site of recurrence, time to recurrence, treatment modality, and the response rates were obtained from the patient files and pathology reports in our gynecologic oncology clinic electronic database system.

Bone scintigraphy, magnetic resonance imaging, and positron emission tomography-computed tomography (PET-CT) were used in the diagnosis of metastatic lesions. For the differential diagnosis of metastasis, systemic examination, chest X-ray, abdominopelvic and thoracic CT and PET-CT were performed. Recurrence that developed only in bone was classified as "isolated recurrence" and bone and other sites were classified as "multiple recurrences". Recurrence of the bone was classified as "axial skeleton", which included cranium, sternum, vertebra and costa, and "appendicular skeleton", which included the upper and lower extremities, scapula, and the pelvic bones. The size of the tumor was defined by the largest diameter of the tumor in the cervix at the initial treatment.

The plan of treatment in the patients with recurrence was decided by the council of gynecology and oncology. Treatment results were evaluated according to the guidelines of the World Health Organization. We defined the clinical response as follows: (I) complete clinical response: disappearance of the macroscopic tumor; (II) partial clinical response: shrinkage over 50% of the macroscopic tumor, (III) stable disease: macroscopic shrinkage of the tumor less than 50% or not less than 25% growth; (IV) progressive disease: more than 25% growth of the macroscopic tumor or macroscopic appearance of new tumor foci[13].

The factors indicating the bone recurrence could not be recognized at this point, because the number of the patients with bone recurrence were only 7 (1.7%). The time of from surgery until recurrence was defined as time-to-recurrence (TTR), the time until the death of the patient was defined as overall survival, and the time of recurrence until the death of patient or until the last date was defined as post recurrence survival.

All patients were followed up after the initial treatment for the CC. Patients who had complete clinical response with salvage treatment for recurrence were followed up quarterly in the first 2 years, semi-annually for up to 5 years, and annually thereafter. Pelvic examination, abdominopelvic ultrasonography, Papanicolaou smear, complete blood count, and biochemistry profile were performed in the follow-up. Chest X-ray was used annually unless there was clinical suspicion. Thoracic and\or abdominal CT were used when needed.

Results

The median follow-up of the main cohort (n=412) was 46 (range=1-300) months. In this period, recurrence developed in 53 (12.9%) patients, and the recurrence rate in bone was observed as 13.2% (7 of 53). Tumor type was squamous carcinoma in six patients and mixed type in one patient (squamous + adenocarcinoma). Paraaortic lymphadenectomy was added to the surgical procedures in six patients and pelvic lymphadenectomy alone was performed in one patient. The number of lymph nodes removed was between 42 and 102. It was determined that there was spread to the pelvic lymph nodes in two patients and pelvic and paraaortic lymph nodes in one patient. There was parametrial invasion in one patient, surgical border positivity in one, and lymphovascular space invasion in two patients. The surgical-pathologic features are shown in Table 1.

One patient (patient #7) received neo-adjuvant chemotherapy. As neoadjuvant chemotherapy, the patient received a combination of cisplatin + tegafur-uracil for 2 cycles. Adjuvant therapy was given to three patients as concurrent chemo-radiotherapy (CCRT) and three patients received no adjuvant therapy. One patient (patient #4) refused adjuvant therapy.

TTR ranged from 9 to 45 months. Five of the recurrences were in the axial skeleton and two were in the appendicular skeleton. Recurrence was observed in three patients in the lumbar vertebrae, one in the thoracic vertebrae, one in the sacral vertebrae, one in the lumbosacral vertebrae, and two in the left femur. Three patients (patient #1, #6, and #7) had isolated bone recurrence and four patients had multiple recurrence. Except for the bone, one of them had it in the inguinal and supraclavicular lymph nodes, one in pelvic-paraaortic lymph nodes, one in lung and paraaortic lymph nodes and one in lung

(Table 2). Recurrence was in the axial skeleton in all isolated bone recurrences.

After recurrence, six patients received salvage therapy for curative intent and one patient received palliative therapy (patient #4). Two of the patients who received salvage therapy were given only systemic treatment (cisplatin + 5 fluorouracil). Four patients received radiotherapy, two of whom were given systemic treatment after radiotherapy. Radiotherapy was performed in one patient with weekly cisplatin (CCRT) and one patient received radiotherapy only (Table 2). In salvage therapy, one patient with only systemic treatment and one patient with radiotherapy had a complete clinical response (patients #1 and #7). These two patients had isolated bone recurrence and their post recurrence survival was 129 months and 11 months, respectively. During the follow-up period, four patients died because of the disease (patient #2, #3, #4 and #5). The recurrence type of these four patients was multiple recurrence, and in two the disease recurred in the lung (Table 2). These patients died within 6-25 months after recurrence. Recurrence was seen in the femur and pelvic paraaortic lymph nodes of the patient who lived up to 25 months after recurrence and was treated with concurrent chemo-radiotherapy.

Discussion

The results of bone recurrence in uterine CC vary widely. Drescher[14] reported bone metastasis in 1.2%. However, this rate was 16% in the study of Fagundes et al.[6]. In our case

Table 1. Clinical, pathologic, and surgical features

Patient no	Age	Histology	Stage	Tumor size (mm)	Lymph node dissection type	Lymphovascular space invasion	Lymph node status/count	Parametrial invasion	Surgical border	Follow-up (month)
1	52	SCC	2A1	40	P-PA	Positive	Positive/1	Negative	Positive	140
2	50	SCC + AC	1B2	45	P-PA	Negative	Positive/10	Negative	Negative	15
3	77	SCC	1B1	35	P-PA	Negative	Negative	Negative	Negative	27
4	61	SCC	1B1	30	P-PA	Negative	Negative	Positive	Negative	44
5	50	SCC	1B1	20	P	Negative	Positive/7	Negative	Negative	54
6	48	SCC	2A1	40	P-PA	Negative	Negative	Negative	Negative	25
7	41	SCC	2A2	60	P-PA	Positive	Negative	Negative	Negative	21

P-PA: Pelvic-paraaortic, P: Pelvic, AC: Adenocarcinoma, SCC: Squamous cell carcinoma, tumor size is the initial size

Table 2. Bone metastasis site, treatment and outcome

Patient no	Adjuvant therapy	TTR (month)	Recurrent bone site	Bone type	Other recurrence site	Salvage therapy for recurrence	Outcome of salvage therapy	Died of disease	Postrecurrence survival (month)
1	CCRT	11	Lumbar vertebra	Axial skeleton	None	Cisplatin + 5FU	Complete clinical response	Negative	129
2	CCRT	9	Femur (left)	Appendicular skeleton	Inguinal LN + Supraclavicular LN	Cisplatin + 5FU	Progression	Positive	6
3	None	9	Femur (left)	Appendicular skeleton	Pelvic-paraaortic LN	CCRT	Progression	Positive	25
4	None[1]	38	Thoracic vertebra	Axial skeleton	Lung + Paraaortic LN	Palliative	Progression	Positive	6
5	CCRT	45	Lumbar vertebra	Axial skeleton	Lung	RT + CT	Progression	Positive	9
6	None	19	Sacral vertebra	Axial skeleton	None	RT + CT	Stable disease	Negative	6
7	None	10	Lumbosacral vertebra	Axial skeleton	None	RT	Complete clinical response	Negative	11

TTR: Time-to-recurrence, CCRT: Concurrent chemoradiotherapy, RT: Radiotherapy, CT: Chemotherapy, 5FU: 5-fluorouracil, LN: Lymph node,
[1]Patient refused treatment

series, the rate of bone recurrence was 1.3% in the main cohort where the median follow-up was 46 months.

The site and number of recurrences are the main factors affecting prognosis and treatment[15]. It is known that the success of treatment is low when recurrence occurs in others site accompanying the bone recurrence[16]. In the current case series, all patients with multiple recurrences died of recurrent disease. The choice of treatment for recurrent disease is primarily dependent on previous treatment and should be evaluated together with the location of the recurrent tumor and the patient's performance[17,18]. In patients with CC who have distant and multiple recurrent disease, the primary aim of treatment is mostly not-curative intent but palliative[19]. However, in a study presented by Makino et al.[20] of 75 patients with uterine CC and bone recurrence, the overall survival (OS) of 16 patients who received chemotherapy and CCRT after RT was 18 months and 2 months, respectively, compared with 25 patients receiving palliative treatment ($p<0.05$). In our case series, complete clinical response was obtained with salvage treatment in two patients in the presence of isolated recurrence. Salvage treatment was applied to one of them with systemic treatment, and with cisplatin and radiotherapy to the other. In recurrent CC, cisplatin is preferred for most patients. Systemic treatment success rate is 12-22% in recurrent CC[21-26]. Unlike other anti-angiogenic agents, bevacizumab has been used as a part of cisplatin-based combination therapy for recurrent, persistent or metastatic CC, and its addition to the cisplatin-paclitaxel protocol has been shown to increase OS from 13 months to 17 months ($p=0.008$)[27,28]. Surgical treatment has been applied in selected cases with solitary bone recurrence in the literature. Ida et al.[29] were able to control the disease by surgical resection in a solitary femur recurrence that developed 22 months after the first treatment. However, Makino et al.[20] reported that in two patients with solitary bone recurrence, complete resection could not be achieved. We had no patients who could be managed surgically in this series.

The retrospective nature is the main limitation of the study. The number of patients was low and this prevented clear results to change clinical practice. However, the study evaluated patients who had a median follow-up of approximately 4 years and who had undergone radical surgery from among more than 400 early-stage cancers.

Conclusion

In conclusion, complete clinical response and long postoperative survival can be achieved with salvage treatment when bone recurrence is solitary. However, the effect of surgery in this patient group should be evaluated. Multimodal treatment options including surgery in CC with bone recurrence, especially solitary recurrence, need to be evaluated in further studies.

Ethics

Ethics Committee Approval: Local ethics committee approval was obtained.

Informed Consent: Approval from all patients.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.Ç., T.T., Concept: C.Ç., R.D., D.Y., Ç.K., Design: C.Ç., Data Collection or Processing: C.Ç., M.Ü., Analysis or Interpretation: C.Ç., Literature Search: C.Ç., G.B., A.K., Ö.M.T., T.T., Writing: C.Ç.

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A spontaneous heterotopic pregnancy presenting with acute abdomen treated with natural orifice transluminal endoscopic surgery procedure: Case report

Doğal orifis ile transluminal endoskopik cerrahi ile tedavi edilen ve akut batın ile başvuran heterotopik gebelik: Olgu sunumu

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Abstract

Heterotopic pregnancy occurs 1 in 30000 pregnancies. We present case of an acute abdomen caused by a ruptured ectopic component. Our patient had no known risk factors, which made the diagnosis even more challenging. Intrauterine pregnancy was desired by patient and her husband. A natural orifice transluminal endoscopic surgery (NOTES) procedure was performed, which is a next-generation minimally invasive procedure in this area. After the procedure, our patient was discharged one day after surgery with a viable intrauterine pregnancy.

Keywords: Heterotopic pregnancy, acute abdomen, natural orifice transluminal endoscopic surgery procedure

Öz

Heterotopik gebelik 1/30000 oranında görülür. Bu olguda hasta rüptüre ektopik komponent sebebi ile oluşan akut batın ile başvurmuştur. Hastanın bilinen bir risk faktörü olmaması tanıyı olduğundan daha zor hale getirmiştir. Hasta ve eşinin intrauterin gebeliği koruma istemi mevcut idi. Hasta bu alanda yeni bir minimal invaziv operasyon tekniği olan doğal orifis ile transluminal endoskopik cerrahi prosedürü ile opere edildi. Post-op 1. günde canlı intrauterin gebelikle taburcu edildi.

Anahtar Kelimeler: Heterotopik gebelik, akut batın, doğal orifis ile transluminal endoskopik cerrahi prosedürü

Introduction

Heterotopic pregnancy is defined as the co-existence of an ectopic pregnancy and an intrauterine pregnancy. This condition occurs in 1 out of 30000 pregnancies of natural conception[1]. Assisted reproductive technologies have a higher rate of 9 out of 10000[2]. A previous history of pelvic inflammatory disease and pelvic surgery is associated with the incidence of heterotopic interstitial pregnancy[3]. Although ectopic pregnancies can be positioned at various anatomic locations such as cervix, ovary, and abdomen, tubal pregnancies have the highest rate of approximately 95-96%[4]. In order to preserve the intrauterine pregnancy, certain clinical manifestations and diagnostic tests alternates compared to ectopic pregnancy.

Transvaginal natural orifice transluminal endoscopic surgery (vNOTES) is a less invasive alternative to laparoscopic salpingectomy. Abdominal cavity is reached through a port placed in the posterior fornix. The safety and efficacy of transvaginal endoscopic salpingectomy for tubal ectopic pregnancy are equivalent to those of laparoscopic procedures. Lesser postoperative pain and a more satisfactory cosmetic outcome were found with the transvaginal endoscopic procedure, making it the more preferred method and superior to the laparoscopic approach[5].

Case Report

This report presents a 29-year-old G1P0 patient who presented to the obstetrics emergency room with severe abdominal pain

PRECIS: Heterotopic pregnancy treated with vNOTES procedure.

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of the left lower quadrant with signs of acute abdomen and knowledge of a 7 weeks' intrauterine pregnancy. The patient was evaluated by the obstetric team upon arrival and her heart rate was 99 bpm, and blood pressure was 90/50 mm Hg. A transvaginal ultrasound was performed, which revealed a 7 weeks and 2 days estimated gestational age intrauterine pregnancy with fetal cardiac activity and was considered viable. Both of the patient's fallopian tubes were dilated and showed evidence of a hydropic fallopian tube, which was evaluated as evidence of former pelvic inflammatory disease. Additionally, a 10.8-cm ectopic pregnancy was positioned on the left side with cardiac activity with an estimated gestational age of 7 weeks and 1 day. Blood products and abdominal free fluid were also detected. At the time, the patient's hemoglobin and hematocrit levels were 12.2 g/dL and 35.4% respectively, and the other initial blood parameters checked were within the normal range (Figure 1, 2).

Assessment

The patient's condition was stabilized with a saline infusion and informed about her clinical condition. The patient expressed a desire to preserve the intrauterine pregnancy. Under close monitoring, the patient was admitted to the gynecology department and was given full information about her condition including the risk of miscarriage and more extensive surgery. After giving consent for the recommended surgery, the patient was taken to the operating room.

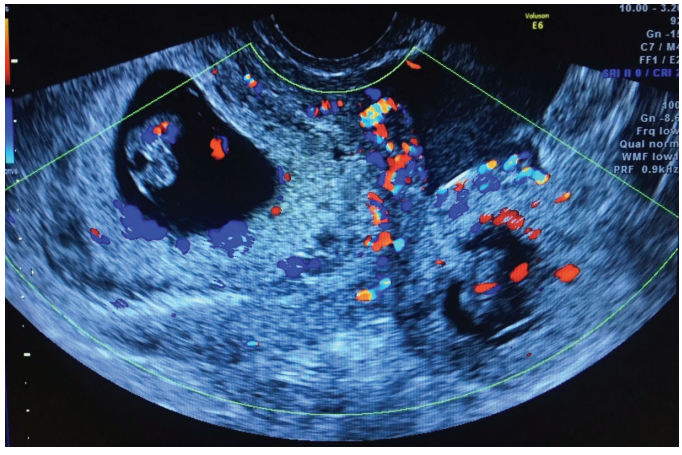

Figure 1. Ultrasound of intrauterine pregnancy and ectopic pregnancy with cardiac activity on Doppler

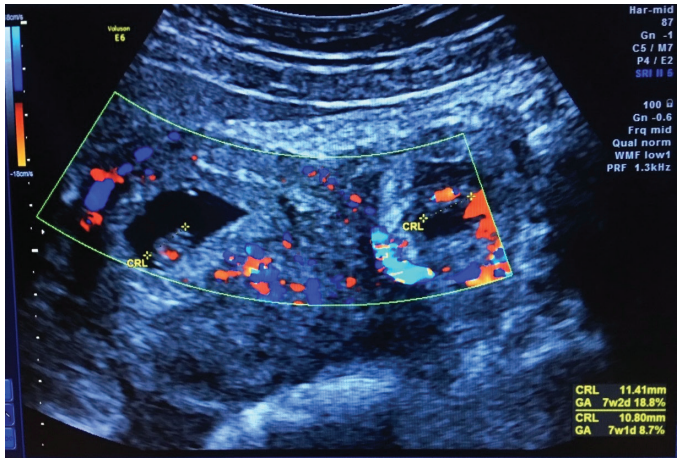

Figure 2. Twin heterotopic pregnancy with estimated gestational age of 7 weeks and 1 day and 7 weeks and 2 days

The patient was placed in the lithotomy position under general anesthesia. Following the sterile covering of the area, posterior colpotomy was used to enter the abdomen. Approximately 200 cc of blood containing clots was drained. The right and left ovary and right fallopian tube were observed in normal anatomic position and nature. The left tube was visualized, which had an approximately 5 cm ectopic mass with active bleeding. After visualizing using a camera, salpingectomy was performed on the left tube using a bipolar energy device. Afterwards, the pelvic area was examined and no bleeding was seen. The colpotomy was closed using sutures. There were no complications.

Follow-up ultrasound was performed in the post-operative 9th hour. The intrauterine pregnancy was detected as viable. Two hundred milligrams of natural progesterone was ordered by vaginal route once the pregnancy was confirmed as viable. On postoperative day 1, the patient's clinical condition and hemoglobin levels were stable and she was discharged. The patient was told to come for a follow-up examination.

One week after discharge, she presented for postoperative follow-ups and screening of the intrauterine pregnancy. Ultrasound showed a live fetus at 8-weeks 0-day gestation based on crown-rump length.

Discussion

The patient who was admitted to our emergency department with acute abdomen and was diagnosed as having heterotopic pregnancy. She underwent salpingectomy with vNOTES and was discharged in good health. Although it is very challenging to diagnose heterotopic pregnancy, high-resolution transvaginal ultrasound is helpful in the process. Yet 20-50% of patients with an interstitial pregnancy present with rupture of the ectopic mass[6]. There are several options for the treatment of heterotopic pregnancy; surgical, medical or expectant treatment. The patient can be treated surgically through salpingectomy or hysterectomy either by laparotomy or laparoscopy. Another option is the direct injection of potassium chloride, hypertonic solution, and methotrexate into the ectopic gestational sac[3,7,8]. Lastly, if the patient has no symptoms and fetal death in gestation is confirmed using ultrasonography, expectant management can be used[9]. The other new way for surgical treatment is vNOTES.

VNOTES is considered to be a next-generation minimally invasive surgical procedure; thus, numerous efforts in this area are being made in many countries. Recently, vNOTES has been performed for cholecystectomy, appendectomy, nephrectomy, and several gynecologic procedures[10-13]. Although NOTES

procedure can be performed through various natural orifices, gynecologists are familiar with the vaginal area; therefore, it has gained more popularity than transanal or transgastric procedures. vNOTES had been performed successfully in a series of surgical procedures including salpingectomy, ovarian cystectomy, myomectomy, hysterectomy, lymphadenectomy, and sacrocolpopexy. Advances in technology have improved the feasibility of vNOTES as a treatment option for gynecologic surgeries. The advantages of vNOTES include reduced postoperative pain, faster post-operative recovery, and decreased postoperative wound infections, as well as outstanding cosmetic results[14]. Access through posterior colpotomy can be challenging if the patient has an adnexal mass or any adhesions causing cul-de-sac obliteration. It is advised to perform a careful vaginal examination before performing this procedure[13]. vNOTES salpingectomy, which can be performed successfully in women diagnosed with ectopic pregnancy, can be used safely in heterotopic pregnancy as seen in our case[15,16]. Although heterotopic pregnancy is quite rare, it should always be in the differential diagnosis of acute abdomen of pregnant patients.

Ethics

Informed Consent: Informed consent was obteined from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.D., C.K., H.C., Concept: K.D., Design: K.D., D.E.I., Data Collection or Processing: D.E.I., İ.K., Analysis or Interpretation: D.E.I., İ.K., Literature Search: K.D., D.E.I., Writing: K.D., D.E.I.

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Immunoglobulin G4 deficiency can be a new entity for primary recurrent miscarriage: Successful pregnancy in two cases after treatment with intravenous immunoglobulin

IgG4 eksikliği, primer tekrarlayan düşük için yeni bir oluşum olabilir: İntravenöz immünoglobulin ile tedavi sonrası iki olguda başarılı gebelik

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Abstract

Recurrent miscarriage is one of the complications of pregnancy in which the potential role of immunologic factors has already been mentioned. Here, two young women with recurrent miscarriage were consulted in the infertility center. The diagnosis of immunoglobulin G4 (IgG4) deficiency was made through the reduction of IgG4 Ig levels and normal total IgG titer. Considering this abnormality, intravenous Ig 200 mg/kg was started monthly, and they both had successful pregnancies. Little is known about IgG4 deficiency in women with recurrent miscarriage. IgG4 deficiency should be taken into account in these patients. It is expected that these results will shed further light on the feasibility of intravenous Ig for women with recurrent miscarriage.
Keywords: Recurrent miscarriage, intravenous immunoglobulin, immunoglobulin G, female infertility

Öz

İmmünolojik faktörlerin potansiyel bir rolünün olduğu belirtilen tekrarlayan düşük, gebeliğin komplikasyonlarından biridir. Burada, tekrarlayan düşüğü olan iki genç kadın infertilite merkezinde değerlendirildi. IgG4 eksikliğinin tanısı, IgG4 immünoglobulin seviyelerindeki ve normal toplam IgG titresindeki düşüşle konuldu. Bu anormallik dikkate alındığında, ayda 200 mg/kg intravenöz immünoglobulin başlandı ve her iki hastada da başarılı gebelikler elde edildi. Tekrarlayan düşüğü olan kadınlardaki IgG4 eksikliği hakkında çok az şey bilinmektedir. IgG4 eksikliği bu hastalarda dikkate alınmalıdır. Bu sonuçların, tekrarlayan düşüğü olan kadınlarda intravenöz immünoglobulinin uygulanabilirliğine daha fazla ışık tutması beklenmektedir.
Anahtar Kelimeler: Tekrarlayan düşük, intravenöz immüglobülin, immünoglobulin G, kadın infertilitesi

Introduction

Recurrent miscarriage (RM) is considered as ≥2 fetal losses before 24 weeks of gestation, affecting an estimated 1-3% of women in their reproductive age. It is classified as primary RM without a previous viable pregnancy and secondary RM with one or more pregnancies[1]. The etiology of RM in up to 50% of cases is genetic. Thyroid disorders have a disputed role in RM, and investigations for thyroid-stimulating hormone and thyroid antibodies are strongly recommended in RM. Searching for uterine malformations should also be considered as a part of diagnostic examinations of couples with RM. There is a doubtful association between hereditary thrombophilia and acquired thrombophilia or Antiphospholipid syndrome with

PRECIS: Heterotopic pregnancy treated with vNOTES procedure

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miscarriage. Antiphospholipid syndrome is diagnosed through the persistent presence of antiphospholipid antibodies and vascular thrombosis; the role of alloimmune mechanisms remains poorly understood[1-3]. The role of immunologic factors has already been mentioned for reproductive failure in patients with RM. A normal pregnancy induces some changes in antibody formation; increased level of immunoglobulin M (IgM) at the early phase of a primary immune response and IgE antibodies in some mothers with a genetic background. At the late phase of primary immune response or after chronic antigen exposure, antibodies switch to IgG; IgG4 in particular have received special attention[4,5].

IgG4 accounts for approximately 5% of total IgG in the serum of adults and is unable to activate the classic complement pathway and antibody-dependent cell-mediated cytotoxicity. IgG4 uniquely plays as a blocking antibody with anti-inflammatory properties; therefore, it is known a as a rule-breaker antibody[6,7]. IgG4 deficiency is the most common immunodeficiency subclass. A deficiency can be identified as a level >2 SD less than age-matched reference values accompanied with a normal total IgG level[8]. Some patients with decreased IgG4 are asymptomatic, but several groups present with recurrent respiratory infections. IgG4 deficiency has been noted in some primary immunodeficiency disorders such as Wiskott-Aldrich syndrome, IgM deficiency, ataxia telangiectasia, and mucocutaneous candidiasis. Other disorders with IgG4 deficiency are Down syndrome, immune thrombocytopenia, lupus erythematosus, and growth hormone deficiency. Monthly intravenous immunoglobulins (IVIG) are the mainstay of treatment for symptomatic patients with IgG subclass deficiencies[9,10]. There are scant data on abnormal Ig subclasses in women with RM[11]. Due to pregnancy loss and its repetitive nature in women, and even its emotional impact on men, there is a need for studies on the etiologic factors of RM. Little is known about IgG4 deficiency in women with RM. We report two young women with primary RM and IgG4 deficiency who became pregnant after IVIG therapy.

Case Reports

Case 1

A 24-year-old Caucasian woman, gravida 2 parity 0, with a history of two miscarriages was seen in Isfahan Fertility and Infertility Center affiliated to Isfahan University after five years of marriage. The ages of miscarriage were 8 weeks and 10 weeks' gestation. She was not a smoker, she had no significant past medical history and was not on any long-term medications. Her physical examination was normal with body mass index (BMI) of 26.6 kg/m^2. Her menstrual cycles were regular (every 28-30 days with 6 days of menstrual flow). The results of hormonal investigations revealed normal gonadotropin levels, implying regular ovulatory cycles. Transvaginal ultrasound of the pelvis showed a normal uterus and ovaries. Based on her history and laboratory features, a diagnosis of unexplained RM was made. Evaluation of blood parameters revealed white blood cells (WBC) 8200 cell/mm^3, hemoglobin (Hb) 14.3 g/dL, and platelets 264000/mm^3. The laboratory tests for diabetes, hyperprolactinemia, and vitamin D deficiency were normal. Antinuclear antibody and anti-dsDNA levels were measured using enzyme-linked immunosorbent assay with a negative result. The other antibody identification studies are shown in Table 1. Considering the history of RM with no causes, further immunologic investigations were performed (Table 2). The detection of low serum IgG4 levels was significant. Treatment with IVIG (Biotest's Intratect®, Germany) was started with 200 mg/kg monthly with the diagnosis of antibody deficiency; she became pregnant after two months. Subsequently, the woman had a successful pregnancy and her infant was delivered at 37 weeks of gestation with a normal Apgar score.

Case 2

A 27-year-old woman with a history of eight miscarriages in the second trimester was referred from Isfahan Fertility and Infertility Center affiliated to Isfahan University. Her age at marriage was 17 years. She had regular menstrual cycles since

Table 1. Different antibody identification in the patients with recurrent miscarriage

Test	Case 1	Case 2
ANA	3.5 U/mL, normal <12 U/mL	8.9 U/mL, normal <12 U/mL
Anti-dsDNA	3.7 U/mL, normal <16 U/mL	2.3 U/mL, normal <16 U/mL
Lupus anticoagulant	39 sec, normal 31-45 sec	negative
Anticardiolipin antibody-lgG	9.6 U/mL, normal <20 U/mL	2.3 U/mL, normal <20 U/mL
Antiphospholipid IgG	0.2 U/mL, normal <12 U/mL	0.1 U/mL, normal <12 U/mL
Anti-SSA (Ro)	1.8 RU/mL, normal <20 RU/mL U/mL	1.4R U/mL, normal <20 RU/mL
Anti-SSB (Ro)	2.2 RU/mL, normal <20 RU/mL	1.7R U/mL, normal <20 RU/mL
Antithyroglobulin antibody	52.7 U/mL, normal <120 U/mL	18.8 U/mL, normal 5-100 U/mL
Antithyroperoxidase antibody	6.37 U/mL, normal 0-40 U/mL	3.0 U/mL, normal 1-16 U/mL

IgG: Immunoglobulin G, ANA: Anti-nuclear antibody

Table 2. Immunologic investigations in the patients with recurrent miscarriage

Test	Case 1	Case 2
Serum IgG	9.0 mg/mL (6.5-18.3 mg/mL)	958 mg/dL (700-1600 mg/dL)
Serum IgM	2.3 mg/dL (0.4-2.6 mg/dL)	132 mg/dL (40-230 mg/dL)
Serum IgA	2.5 mg/mL (0.7-3.6 mg/mL)	350 mg/dL (70-400 mg/dL)
CD3 + T cells	73% (58-86%)	70% (68-82%) of lymph
CD3 + CD4 + T cells	31 (32-64%)	31 (28-58%)
CD3 + CD8 + T cells	38 (13-40%)	43 (19-48%)
CD3+ CD4 +/ CD3 + CD8 +	0.8*	0.7*
CD16 + NK cells	15% (5-19%)	9% (6-29%)
CD56 + NK cells	25% (3-25%)	17% (6-29%)
Serum IgG1	4.1 mg/dL (3.1-8.5 mg/dL)	5.7 mg/dL (2.2-5.4 mg/dL)
Serum IgG2	4.0 mg/dL (0.6-4.9 mg/dL)	1.8 mg/dL (0.2-1.9 mg/dL)
Serum IgG3	0. 9 mg/dL (0.2-1.9 mg/dL)	0.9 mg/dL (0.06-1.1 mg/dL)
Serum IgG4	<0.05 mg/L (0.1-1.0 mg/L)*	0.05 mg/L (0.1-1.0 mg/L)*

Ig: Immunoglobulin, *Significant level

the age of 13 years. There was no history of smoking and other diseases. At the time of her referral, her BMI was 26.8 kg/m^2. Transvaginal ultrasound revealed no anomalies of the uterus and ovaries. The initial complete blood count revealed WBCs of 10.300 cell/mm^3, Hb of 12.9 g/dL, and platelets of 35.7000/mm^3. Laboratory investigations were performed to exclude diabetes mellitus and prolactin deficiency. The levels of antibodies in the serum are shown in Table 1. Nephelometry was used to determine the total levels of IgG subclasses. The immunologic profile abnormalities including low levels of serum IgG4 and decreased ratio of CD4 to CD8 are shown in Table 2. Considering the IgG4 deficiency, we started IVIG 200 mg/kg monthly; the patient is now pregnant with gestational age of 32 weeks.

Discussion

This study presents two women with low levels of IgG4 and primary RM. After treatment with IVIG, they had successful pregnancies. To date, there have been very few reports of IgG4 deficiency in women with RM[12]. A normal pregnancy is associated with increased levels of interleukin (IL)-10 and IL-4 related to Th2 type, whereas some miscarriages are associated with high levels of interferon-gamma and IL-12 related to Th1 type. In normal pregnancy, secretion of IL-4 causes the plasma cells to switch to the production of IgG4, whereas in miscarriage, elevated levels of interferon-gamma inhibits the process of switch to IgG4[4]. Wilson et al.[11] study showed that women with RM had significantly low levels of total IgG and IgG subclasses 1, 2, 3, and 4 compared with normal pregnant women. IgG4 is an antibody with unique biologic properties[13]. There is evidence for the beneficial effect of IgG4 in allergic disease by inhibiting mast cell degranulation and impairing anti-tumor immunity in malignant melanoma; however, the mechanism of IgG4 in normal pregnancy is not fully understood. IgG4 generally induces a tolerance mechanism in allergic immunotherapy; therefore, it may play an important role in maternal tolerance to the fetus and maintenance of pregnancy. A new clinical study by Piccinni et al.[14] suggests that the local production of leukemia inhibitory factor (LIF) is necessary for embryo implantation and it can be up-regulated by IL-4 and progesterone. We think IgG4 may contribute to LIF production; low level of IgG4 followed by miscarriage. The level of IgG4 in our two cases was very low. It is notable that as many as 10-15% of normal people have IgG4 concentrations below the limit of detection[15]. A prominent feature in the CD markers of our patients was the decreased the ratio of CD3CD4 T cells to CD3CD8 T cells. A normal CD4:CD8 ratio in pregnant adults is not 2:0; Ghafourian et al.[16] investigated different types of T-cell subsets in 25 women with RM. Compared with normal, the proportion of CD3CD8 T cells was significantly higher in women with RM. IVIG was administered for the treatment of our cases with successful results, similar to Abdou et al.[17] study. The mechanism of IVIG in the treatment of women with RM is the modulation of immune cells. A recent study found that IVIG increased the regulatory T cells and diminished Th 17 responses in RM[18]. Although we did not measure post-treatment IgG4 levels in our patients, it could explain the possible beneficial effect of IVIG on the increased levels of IgG4 in women with RM and deficiency of IgG4. We recommend IgG4 subclass determination in the initial evaluation of women with RM. We need a deeper understanding of the role of IgG4 in pregnancy; it is expected that these results will shed further light on the feasibility of IVIG for women with RM.

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Ethics

Informed Consent: Was taken.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.S., N.H., Concept: B.S., N.H., Design: B.S., N.H., Data Collection or Processing: B.S., N.H., M.M., Analysis or Interpretation: B.S., N.H., M.M., Literature Search: M.M., B.S., Writing: M.M.
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Letter to Editor

Letter to Editor

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Keywords: Neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, obstetric
Anahtar Kelimeler: Nötrofil/lenfosit oranı, platelet/lenfosit oranı, obstetri

To the Editor,

We read the article of Jaffar DW. and Rabie MAF. titled "Maternal platelet-to-lymphocyte ratio at delivery can predict poor neonatal outcome in preterm births" published in 15(4)p.254,2018. In recent years, intensive research has been conducted on the use of hematologic inflammatory parameters such as the neutrophil/lymphocyte ratio and the platelet/lymphocyte ratio (PLR) in obstetric and gynecologic pathologies. On the other hand, there are few studies showing the effect of these parameters on neonatal outcomes. Therefore, we think that the results of this study are very important. PLR is associated with maternal immune activation and is therefore thought to increase more in inflammation-related processes such as pre-eclampsia and preterm labor[1]. In the materials and methods section of this study, we observed that the obstetric characteristics of the pregnant women group were not specified. It is not stated whether any of the 439 preterm labor cases in the study included early membrane rupture. It is also not indicated whether some subjects were excluded due to pregnancy-induced hypertension or pre-eclampsia. It is known that pre-eclampsia produces a maternal systemic inflammatory response, neutrophils increase and lymphocytes decrease[2]. Similarly, inflammation has been implicated in the etiology of early membrane rupture cases[3]. In the literature, the results studies evaluating the effect of PLR on neonatal outcomes vary[4,5]. The variable results can be attributed to maternal obstetric conditions (such as preterm labor, pre-eclampsia) and the different source of the blood sample (maternal/fetal). For these reasons, the diagnosis of patients, their hospitalization, status of thrombocytopenia due to pregnancy, and medical treatments should be specified. It should also be explained by which indication, in what manner, the number of gestational weeks at which they gave birth, and when the hemogram samples were taken. As can be expected, antibiotic, steroid applications, and developing obstetric complications may change the hemogram parameters. For these reasons, clarification of the materials and methods section of the article will provide a healthier evaluation of the results.

Ethics

Informed Consent: Was obtained.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: D.A.K., Design: T.O., Data Collection or Processing: M.D.Ç.,Analysis or Interpretation: E.B., Literature Search: T.O., Writing: D.A.K.,
Conflict of Interest: No conflict of interest was declared by the authors.
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