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

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

June 2018 Volume: 15 Issue: 2 www.tjoddergisi.org

TURKISH SOCIETY OF OBSTETRICS AND GYNECOLOGY

galenos yayınevi

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Printing at: Özgün Ofset Ticaret Ltd. Şti.
Yeşilce Mah. Aytekin Sk. No: 21 34418 4. Levent, İstanbul, Turkey
Phone: +90 (212) 280 00 09
Printing Date: June 2018
ISSN: 2149-9322 **E-ISSN:** 2149-9330
International scientific journal published quarterly.

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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Turkish Journal of Obstetrics and Gynecology (formerly called Türk Jinekoloji ve Obstetrik Derneği Dergisi) is the official peer-reviewed publication of the Turkish Society of Obstetrics and Gynecology and is published quarterly on March, June, September and December.

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The "Turkish Journal of Obstetrics and Gynecology" is the official publication of the Turkish Society of Obstetricians and Gynecologists. The journal is published quarterly (March, June, September and December) in English and publishes original peer-reviewed articles, reviews, case reports and commentaries in the fields of gynecology, gynecologic oncology, endocrinology and reproductive medicine and obstetrics. The journal gives publication priority to original research articles over case reports. Reviews are considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer-reviewed journals on the topic of the review and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer-review from qualified experts in the area.

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· The declaration of transparency from the corresponding author.

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· Institutional review board (IRB) approval or exemption.

· Informed consent.

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

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

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."

Conclusion

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

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Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research.

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

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References are numbered (Arabic numerals) consecutively in the order in which they appear in the text (note that references should not appear in the abstract) and listed double-spaced at the end of the manuscript. The preferred method for identifying citations in the text is using within parentheses. Use the form of the "Uniform Requirements for Manuscripts" (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/). If number of authors exceeds seven, list first 6 authors followed by et al.

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Revisions will be sent to the corresponding author. Revisions must be returned as quickly as possible in order not to delay publication. Deadline for the return of revisions is 30 days. The editorial board retains the right to decline manuscripts from review if authors' response delays beyond 30 days. All reviewers' comments should be addressed a revision note containing the author's responses to the reviewers' comments should be submitted with the revised manuscript. An annotated copy of the main document should be submitted with revisions. The Editors have the right to withdraw or retract the paper from the scientific literature in case of proven allegations of misconduct.

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LETTER FROM THE PRESIDENT

Dear Colleagues,

We are delighted to announce you that our 16th National Congress of Obstetric and Gynecology was done with the attendance of 1700 participants who were satisfied with current scientific and social sessions.

For the last 2 years, I am working on a project in order to decrease maternal mortalities all over the country. I am mentioning especially "fear of our colleagues" from litigation or violence from patients/patients' relatives. And I am saying "no deal to fears at medicine!", we cannot do our works with these threats. For this issue I visited more than 20 cities and listened my colleagues. I realized that the main issue is the cooperation between the colleagues! If they support each other in case of an emergency situation or in a conflict with patients, the percentage of litigation and violence towards doctors would be decreasing. Since saving our patients lives needs safety and protection we need firstly cooperation with each other. I will continue to work on this "colleague cooperation support!"

In medicine, producing new and reliable datas are the most important issue for all over the world. We want to take a significant, strong and reliable part of this scientific world as the Turkish Obstetrics and Gynecology Journal with your supports. We are waiting your precious papers to our journal.

Sincerely,

Ateş Karateke, Prof. MD
President of TJOD

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EDITORIAL

Dear Coleagues,

According to Web of Science database we have received 22 citations between 2015 and 2018. The citation number is 24 in Scopus database. Total full text article requests, PDF downloads are gradually increasing December 2017; 4798, January 2018, 5836, February 2018, 6461. Mostly requested articles are reviews and researches in the field of obstetrics.

As the Turkish Society of Obstetrics and Gynecology is trying to improve surgicals skills of its members we organize a series of cadaveric courses again in 2018: on 29 June-1 July Laparoscopy and hysteroscopy course, on 6-7th of July Cosmetic Gynecological Surgery, on 21-22 of July Hands on Surgery on Cosmetic Gynecology, 31st of August European Society of Gynecological Oncology endorsed vulvar cancer surgery cadeveric workshop, on 1-2 of September abdominal gynecological oncology approach. All courses will be held in conjunction with Bahçeşehir University. We are following the collegues for 1-2 year if they could apply what they have learned in these courses.

In this issue of we have two comprehensive reviews on "Aesthetic Gynecology" and "In vitro Maturation". There is also urgent need about Turkish women's genital perception and the view of husbands on these issues. We are looking forward for more studies on this topic in Turkey.

Best wishes

Eray Çalışkan
Editor in Chief

Clinical Investigation / Araştırma

DOI: 10.4274/tjod.43931

Turk J Obstet Gynecol 2018;15:70-4

Postpartum urinary retention: Evaluation of risk factors

Postpartum üriner retansiyon: Risk faktörlerinin değerlendirilmesi

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Abstract

Objective: Postpartum urinary retention means the absence of spontaneous micturition more than 6 hours after birth or when residual volume after urination is less than 150 cc. If neglected, postpartum urinary retention may result in bladder denervation and detrusor muscle weakness requiring intermittent catheterization or permanent micturition dysfunction. Our goal was to identify the possible risk factors for postpartum urinary retention.
Materials and Methods: Five hundred sixty female subjects were included in this retrospective study. All data obtained including variables such as age, parity, body mass index, duration of labor, prepartum bladder catheterization were compared between female subjects with and without postpartum urinary retention.
Results: Among the 560 patients recruited to our study, 124 (22.1%) had postpartum urinary retention. Third stage duration, time from birth to the first void, and number of peripartum micturitions were found to be potential risk factors for postpartum urinary retention. Different than other studies, our study revealed a correlation between peripartum catheterization and postpartum urinary retention. There were no statistically significant differences between patients with and without postpartum urinary retention in terms of other variables.
Conclusion: In this study, a correlation between peripartum catheterization and postpartum urinary retention was found. There are studies that reported the possible risk factors related to the occurrence of postpartum urinary retention. More studies should be conducted to investigate long-term results with larger populations.
Keywords: Postpartum urinary retention, postpartum bladder dysfunction, risk factors

Öz

Amaç: Postpartum üriner retansiyon; doğum sonrası 6 saatlik bir sürede miktürasyonun kendiliğinden olmaması veya rezidüel idrar volümünün >150 cc'den fazla olması olarak tanımlanır. İhmal edilen postpartum üriner retansiyon, mesane denervasyonuna ve aralıklı kateterizasyon gerektiren detrusor atonisine veya kalıcı işeme disfonksiyonu neden olabilir. Amacımız, doğum sonrası üriner retansiyonun olası risk faktörlerini tanımlamaktır.
Gereç ve Yöntemler: Bu retrospektif çalışmaya 560 hasta dahil edildi. Yaş, parite, vücut kitle indeksi, doğum süresi, doğum öncesi mesane kataterizasyonu gibi değişkenler dahil olmak üzere elde edilen tüm veriler postpartum üriner retansiyonu olan ve olmayan hastalar arasında karşılaştırıldı.
Bulgular: Çalışmamızda 560 hastadan 124'ünde (%22,1) postpartum üriner retansiyon saptandı. Postpartum üriner retansiyon için üçüncü evre süresi, doğumdan ilk işemeye kadar geçen süre, peripartum miktürasyon sayısı olası risk faktörleri olarak saptandı. Çalışmamız, diğer çalışmalardan farklı olarak, peripartum kateterizasyon ile postpartum üriner retansiyon arasındaki korelasyonu ortaya koymuştur. Postpartum üriner retansiyonu olan ve olmayan hastalar arasında diğer değişkenler açısından istatistiksel olarak anlamlı farklılık bulunmadı.
Sonuç: Bu çalışmada, peripartum kateterizasyon ile postpartum üriner retansiyon arasında korelasyon saptandı. Postpartum üriner retansiyon oluşumuyla ilişkili olası risk faktörlerini bildiren çalışmalar bulunmaktadır. Uzun vadeli sonuçları araştırmak için geniş popülasyonlarla daha fazla çalışma yapılmalıdır.
Anahtar Kelimeler: Postpartum, postpartum üriner retansiyon, mesane disfonksiyonu, risk faktörleri

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

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Received/Geliş Tarihi: 10.12.2017 **Accepted/Kabul Tarihi:** 18.03.2018

Introduction

Postpartum voiding dysfunction is defined as having difficulty in complete micturition leading to urine retention or an inability to urinate spontaneously after giving birth[1]. The exact incidence is not clear because of undiagnosed asymptomatic cases, but according to the literature, the estimated incidence of postpartum urinary retention (PPUR) has a wide range between 0.05% and 37% depending on the definitions used[2]. Overt PPUR is defined as the failure in spontaneous voiding within six hours of vaginal birth, whereas covert PPUR refers to a bladder volume of ≥150 mL remaining after spontaneous urination[3]. Although PPUR has an uncertain pathophysiology, there are several hypotheses on the cause of PPUR. Vaginal delivery can be traumatic for pelvic floor muscles and innervations, which can cause reduced bladder sensitivity. Also, peri-urethral and vulvar edema due to vaginal delivery may result in obstruction. Many prognostic factors have been identified such as mode and duration of labor, the presence of perineal trauma, the method of anesthesia or analgesia, the body mass index (BMI) of the patient, and the birth weight of the baby. The long-term effects of PPUR have not yet been identified, but many complications of persistent urinary retention due to non-delivery causes have been described. If neglected, urinary retention may result in denervation of the bladder, detrusor muscle weakness and failure, anuria, hydronephrosis, and even kidney failure caused by renal obstruction[4,5]. Whether specific treatment is unnecessary or requires intermittent catheterization may be self-limiting[6]. Screening for PPUR is not part of standard postpartum care; therefore, identifying the risk factors and early diagnosis are as important as appropriate management to prevent the potential damage of enduring retention. Our aim was to describe the possible risk factors for postpartum bladder dysfunction.

Materials and Method

We conducted a retrospective study of 560 women who have birth from January 2014 to September 2016 at a single tertiary center. The study was approved by the Ethics Committee of University of Health Sciences, Diyarbakır Gazi Yaşargil Training and Research Hospital in Turkey (approval number: 2018-30). Approval of the local ethics committee was provided for the study and the study protocol adhered to the principles of the Declaration of Helsinki. Patients who were older than 18 years and delivered via vaginal birth were included in the study. Patients who had urinary tract disease, overactive bladder, pelvic organ prolapsus or previous bladder surgery were excluded. Demographic data such as age, parity, BMI, and obstetric variables such as the use of augmentation with oxytocin or Propess®, duration of active phase of labor, presence of episiotomy, degree of laceration, birth weight of the baby, number of voids during delivery, and peripartum bladder catheterization were documented and analyzed. The patients were considered as having PPUR when there was no micturition within 6 hours of delivery. For patients who were suspected as having PPUR immediately after the first micturition, residual urine volume was measured using transabdominal ultrasonography (USG) (Logiq Pro 200, 3.5 MHz convex transducer probe) and these data were documented from the patients' medical files. Estimated post voiding bladder volume was calculated using the formula, D1xD2xD3x0.9 (D1: the widest diameter in the transverse scan, D2: anteroposterior diameter in the longitudinal scan, D3: cephalocaudal diameter in the longitudinal scan)[7]. Assessment of urinary retention during the peripartum period is difficult and there is no consensus on the amount of bladder volume that is considered as urinary retention. Generally, a normal post-micturition residual volume is accepted as 50-200 mL in women[8]. Bladder volume can be measured by catheterization with considerable risk of urinary infection. Transabdominal USG is a non-invasive method for the assessment of urinary retention but its accuracy may be limited especially during prepartum because of the fetus[9]. However, in this study, USG was used without this limitation because PPUR was assessed.

We defined PPUR as a failure in spontaneous voiding within six hours of birth or post void bladder volume >150 mL in accordance with the literature, as verified using USG[10]. All obtained data were compared between women with and without PPUR.

Statistical Analysis

The statistical analyses were performed using the Statistical Package for the Social Sciences software, Version 17.0. Categorical data were compared using the chi-square test, Fisher's exact test, and Student's t-test for normal distribution, or the Mann-Whitney U test for those with non-normal distribution. $P<0.05$ was considered statistically significant. Significant variables were analyzed by bivariate and multivariate logistic regression analyses to determine which factors were independently associated with PPUR. Odds ratio and corresponding 95% confidence intervals are reported.

Results

Among the 560 patients recruited to our study, the mean ages of patients with and without PPUR were 27.90±6.69 years and 27.19±6.83 years, respectively. The incidence of PPUR was 22.1% (n=124). There were no statistically significant differences between patients with and without PPUR regarding age, BMI, gravida, parity, induction with oxytocin or Propess®, use of vacuum or forceps, duration of the second and third stage, fundal pressure, peripartum catheterization, number of micturitions, post-micturition residual volume, birth weight of the baby, presence of episiotomy or deep perineal laceration, and duration of the active phase. Table 1 describes the demographic data of women and labor characteristics with and without PPUR. When compared with patients without PPUR, the duration of the third stage was longer in women with PPUR (13.57±10.31 minutes vs. 17.02±12.93 minutes). The number of peripartum

micturitions was significantly higher in women without PPUR than patients with PPUR (p<0.001). The analyses showed that the time from birth to the first void was significantly longer in women with PPUR (661.02±1372.77 minutes vs. 212.57±80.00 minutes). No significant differences were found in terms of other variables. When the factors affecting the development of PPUR were analyzed, it was shown that a 10 minute increase in the second stage of labor led to a 6% increase in the risk of PPUR; a 1 minute increase in time from birth to the first void increased PPUR risk by 4%. The absence of prepartum bladder catheterization was related to 2.2-fold increased risk of PPUR development. Given the effect of the number of peripartum voids on PPUR, an increase of one in the number of voids reduced the risk by 24.1%. No statistically significant effect of other variables on the occurrence of PPUR was found (Table 2).

Table 1. Characteristics of patients and labor with or without postpartum urinary retention

	PPUR (-) (n=436)	PPUR (+) (n=124)	p
Age	27.90±6.69	27.19±6.83	0.298*
BMI	27.16±4.99	26.74±5.24	0.430*
BMI group Normal Overweight Obese	 156 (35.8%) 155 (35.6%) 125 (28.7%)	 46 (37.4%) 49 (39.8%) 28 (22.8%)	0.412‡
Gravida median (IQR)	2 (3)	2 (3)	0.606†
Primiparous/ nulliparous	289/147	76/47	0.355‡
Induction with oxytocin	191 (43.8%)	49 (39.8%)	0.432‡
Induction with Propess®	70 (16.1%)	19 (15.4%)	0.871‡
Augmentation	227 (52.1%)	61 (49.6%)	0.628‡
Duration of active phase (min)	170.51±148.57	176.90±130.91	0.177†
Duration of the second stage (min)	34.16±37.05	46.25±55.09	**0.055**†
Fundal pressure	204 (46.8%)	58 (47.2%)	0.943‡
Forceps	23 (5.3%)	8 (6.5%)	0.599‡
Vacuum	9 (2.1%)	2 (1.6%)	0.551§
Deep perineal laceration	22 (5.0%)	10 (8.1%)	0.193‡
Episiotomy	266 (61.0%)	73 (59.3%)	0.739‡
Peripartum catheterization	242 (55.5%)	45 (36.6%)	**0.001**
Duration of the third stage (min)	13.57±10.31	17.02±12.93	**0.001**†
Number of micturitions (min-max)	2 (0-10)	2 (0-6)	**0.001**†
Birth weight (grams)	3228±464	3203±453	0.598*
Postmicturition residual volume (mL)	81.74±112.05	141.32±142.22	**0.001**†
Time from birth to first void (min)	212.57±80.00	661.02±1372.77	**0.001**†

*: t-test for independent samples, †: Mann-Whitney U test, ‡: Chi-square test, §: Fisher's exact test, BMI: Body mass index, IQR: Interquartile range, PPUR: Postpartum urinary retention, Min: Minimum, Max: Maximum

Discussion

We found that PPUR was relatively common with an incidence of 22.1%. The reported incidence in the literature varies between 0.05-37%[2,11]. These differences may be due to

Table 2. Factors affecting the development of postpartum urinary retention (bivariate logistic regression)

	OR	95% CI	p
Age	0.984	0.954-1.015	0.298
BMI Overweight Obese	 1.072 0.760	 0.677-1.698 0.449-1.285	 0.767 0.305
Gravida	1.005	0.911-1.108	0.925
Nulliparity	1.216	0.803-1.840	0.355
Induction with oxytocin	0.849	0.565-1.277	0.432
Induction with Propess®	0.955	0.550-1.659	0.871
Duration of active phase (min)	1.0001	0.999-1.002	0.666
Duration of the second stage (min)	1.006	1.002-1.100	0.007
Fundal pressure	1.015	0.680-1.515	0.943
Deep perineal laceration	1.665	0.767-3.618	0.198
Episiotomy	0.933	0.620-1.403	0.739
Prepartum bladder catheterization	2.162	1.431-3.267	0.001
Duration of the third stage (min)	1.025	1.008-1.042	0.003
Number of peripartum voids	0.759	0.657-0.877	0.001
Birth weight (grams)	1.0001	0.999-1.0001	0.598
Post-voiding residual volume	1.004	1.002-1.005	0.001
Time from birth to first void (min)	1.103	1.068-1.140	0.001

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, Min: Minimum, Max: Maximum

different study designs and under diagnosis of covert retention. However, many of the cases remain undiagnosed and most patients with PPUR have no symptoms. The average maternal age ranges between 25 and 28 years in the literature[12]. In our study, the mean age was also within these ranges for patients with and without PPUR (27.90±6.69 years, 27.19±6.83 years, respectively). The pathophysiology of PPUR is still unclear. Some physiologic, neurologic, and mechanical causes may be responsible for PPUR development. The detrusor muscle can be inhibited by the effect of increased progesterone level, thereby leading to urinary retention. Vaginal delivery can be traumatic for pelvic floor muscles and innervations, which can result in hypotonicity or reduced bladder sensitivity. Also, peri-urethral and vulvar edema due to vaginal delivery may result in obstruction[13,14]. In the literature, many risk factors have been identified for the occurrence of PPUR. The development of PPUR may be caused by prolonged stages of delivery. We found that prolonged second and third stage of labor increased the risk of PPUR, but the duration of the active phase was not associated with PPUR. A possible mechanism is applied mechanical strength, which contributes to pelvic nerve damage leading to neurologic impairment of the bladder. In some studies, the association of prolonged labor with PPUR has also been reported[6,12]. Similarly, in a study conducted by Kekre et al.,[3] it was reported that PPUR rates were higher in patients with a prolonged second stage of labor. Salemnic et al.[6] claimed that there was a relationship between PPUR and longer second stage of birth and mediolateral episiotomy, yet this study was performed on 200 women who delivered vaginally and only once. In a larger study conducted by Yip et al.,[12] only women with vaginal deliveries (n=691) were studied and it was found that a duration of delivery more than 800 minutes was significantly correlated with a rise in PPUR. In the present study, we found no relationship between parity and PPUR development. Nulliparity is usually perceived as a risk factor[1,14]. Nulliparous women are thought to be exposed to pelvic floor tenderness and pudendal nerve damage in the course of vaginal birth. The risk of developing PPUR was higher in primigravida than multigravida women in a study conducted by Liang et al.[15]. In contrast to our findings, Pifarotti et al.[16] reported that fundal pressure was another risk factor for PPUR. Unlike our study, many studies reported that perineal damage had an impact on the development of PPUR[17,18]. A study by Musselwhite et al.[17] revealed that second- and third-degree perineal tears, which might result in reflex urethral spasm, had a relationship with PPUR. However, in the same study, episiotomy was found to have no impact on PPUR[17]. The risk of PPUR was increased by perineal tears including sphincter rupture in a study conducted by Glavind and Bjork[18]. In contrast, Yip et al.[12] reported that perineal trauma had no effect on the incidence of PPUR.

Contrary to other authors[3,19], we detected no association between vaginal operative birth and PPUR. Vaginal operative delivery can be confused with other factors such as longer delivery, epidural analgesia, parity and episiotomy, and causes debates regarding whether it is an independent predictor[1]. Several studies, as well as ours, have shown that fetal birth weight does not increase the risk of PPUR[8,20]. Conversely, it has been reported that PUR was also due to infant birthweight lower than 3800 g[14]. In our study, we did not document the follow-up of the patients. In a study by Carley et al.,[11] 11.332 women were evaluated following vaginal delivery and it was found that 45% of symptomatic PPUR resolved within 48 hours and 25% of women had persistence for more than 72 hours. Various short-term complications of PPUR have been identified, but it is not certain whether PPUR is related to any long-term morbidity. Women with PPUR were not significantly different from those without PPUR in the study by Yip et al.,[21] which was a four-year follow-up about women with PPUR, using outcome variables such as urinary stress incontinence, fecal incontinence, frequency, nocturia, urgency, urge incontinence, and coital incontinence. In a larger study, it was reported that 0.05% of PPUR had persisted related to long-term bladder dysfunction[19]. There is no consensus on the significance of PPUR[22]. Furthermore, guidelines do not recommend routine bladder scanning for the diagnosis of PPUR, due to the size of the uterus after delivery, and the accuracy of USG measurements of residual volume is debatable[23,24]. More research is needed to confirm the benefits and cost-effectiveness of post-routine bladder screening. The present study demonstrates that the PPUR development rate is lower in patients who had peripartum bladder catheterization. There are many studies in the literature reporting the possible risk factors related to the occurrence of PPUR, but most do not mention peripartum bladder catheterization, which makes it difficult to compare them with results in terms of the effect of catheterization on the development of PPUR. To the best of our knowledge, this is the first study to define the relationship between peripartum catheterization and the development of PPUR.

Study Limitations

One of the limitations of the study is it's retrospective and the small number of cases. Another point is that it did not include volumes of voiding or oral intake of the women.

Conclusion

In conclusion, PPUR is a relatively common problem that can lead to irreversible damage to the bladder. Further prospective studies with more participants are needed to support the findings and define long-term implications of PPUR for detecting, avoiding, and managing postpartum voiding dysfunction.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University of Health Sciences, Diyarbakır Gazi Yaşargil Training and Research Hospital in Turkey (approval number: 2018-30).

Informed Consent: Consent form was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.P., O.D., Concept: M.P., M.B.Ş., Design: Ç.P., O.D., Data Collection or Processing: M.B.Ş., Ç.K., M.Ş.B., Analysis or Interpretation: O.D., M.Ş.B., Literature Search: M.P., O.D., Ç.K., Writing: Ç.P., O.D.

Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.57255

Turk J Obstet Gynecol 2018;15:75-9

Evaluation of the diabetes in pregnancy study group of India criteria and Carpenter-Coustan criteria in the diagnosis of gestational diabetes mellitus

Gestasyonel diabetes mellitus tanısında Hindistan kriterleri ve Carpenter-Coustan kriterleri ile gebelik çalışma grubunda diyabetin değerlendirilmesi

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Abstract

Objective: Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance that is diagnosed for the first time during pregnancy. This prospective study was undertaken to validate the single-step non-fasting 75 gm Diabetes in Pregnancy Study Group of India (DIPSI) criteria of GDM in Indian patients in comparison with the two-step fasting 100 gm glucose challenge through the Carpenter Coustan criteria (CCC).
Materials and Methods: Two hundred patients underwent comparative testing using the DIPSI criteria and CCC. Plasma venous blood glucose levels were estimated using the hexokinase method; values ≥140 mg/dL at 2 hours were considered positive according to the DIPSI criteria. Any two values from ≥95 mg/dL for fasting, ≥180 mg/dL at 1 hour, ≥155 mg/dL at 2 hours, and ≥140 mg/dL at 3 hours were considered positive with the CCC.
Results: The mean age and body mass index were 24.26±3.75 years and 20.7±3.07 kg/m^2. The sensitivity, specificity, and positive and negative predictive values of the DIPSI guidelines were found as 100%, 97.14%, 83.87%, and 100%, respectively. The positive and negative likelihood ratios were 35.8 and zero. Diagnostic accuracy was found as 97.56%.
Conclusion: DIPSI having high sensitivity, specificity, negative predictive value and diagnostic accuracy. DIPSI offers simplicity, feasibility, convenience, and repeatability while economizing universal screening and diagnosis of GDM on a mass-scale. The DIPSI procedure has the potential to be applied to the entire obstetric population, in the implementation of public health programs to diagnose GDM in the community, thus reaching the needs of the developing world.
Keywords: Gestational diabetes mellitus, Diabetes in Pregnancy Study Group of India criteria, Carpenter Coustan criteria, pregnancy, glucose tolerance test

Öz

Giriş: Gestasyonel diabetes mellitus (GDM) gebelik sırasında ilk kez teşhis edilen herhangi bir glukoz intolerans derecesi olarak tanımlanır. Bu prospektif çalışma Hintli hastalarda; tek aşamalı, tok, 75 g glukoz yüklemesine dayanan Hindistan'da Hamilelikte Diyabet Çalışması Grubu (DIPSI) kriterlerinin, iki aşamalı, aç, 100 g glukoz yüklemesine dayanan Carpenter Coustan kriteri (CCK) kriterleri ile karşılaştırılarak doğrulanması için yapılmıştır.
Gereç ve Yöntemler: İki yüz hastaya DIPSI kriterleri ve CCK kullanılarak karşılaştırmalı test uygulandı. Plazma venöz kan glukoz düzeyleri hekzokinaz yöntemi kullanılarak hesaplandı; DIPSI kriterlerine göre 2 saatte 140 mg/dL'nin üstündeki değerler pozitif olarak değerlendirildi. Açlık için ≥95 mg/dL, 1 saatte ≥180 mg/dL, 2 saatte ≥155 mg/dL ve 3 saatte 140 mg/dL olmak üzere herhangi iki değer CCK ile pozitif olarak değerlendirildi.
Bulgular: Ortalama yaş 24,26±3,75 yıl ve beden kitle indeksi 20,7±3,07 kg/m^2 idi. DIPSI kılavuzlarının hassasiyeti, özgüllüğü, pozitif ve negatif öngörü değerleri sırasıyla %100, %97,14, %83,87 ve %100 bulundu. Pozitif ve negatif öngörü oranları 35,8 ve sıfır idi. Tanı doğruluğu %97,56 olarak bulundu.
Sonuç: DIPSI; yüksek hassasiyete, özgüllüğe, negatif öngörü değerine ve tanısal doğruluğa sahiptir. Kitlesel ölçekte, GDM tanısını ve evrensel taramayı hızlandırırken basitlik, fizibilite, rahatlık ve tekrarlanabilirlik sunar. DIPSI prosedürü; toplumda GDM'yi teşhis etmek için halk sağlığı programlarına, tüm obstetrik popülasyona uygulanabilecek potansiyele sahip olup, böylece gelişmekte olan dünyanın ihtiyaçlarına ulaşmaktadır.
Anahtar Kelimeler: Gestasyonel diabetes mellitus, Hindistan'da Hamilelikte Diyabet Çalışması Grubu kriterleri, Carpenter Coustan kriterleri, gebelik, glukoz tolerans testi

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Received/Geliş Tarihi: 15.01.2018 **Accepted/Kabul Tarihi:** 18.03.2018

PRECIS: Diabetes in pregnancy study group of India criteria versus carpenter coustan criteria for diagnosing gestational diabetes mellitus.

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance that is diagnosed for the first time during pregnancy, irrespective of treatment with diet or insulin[1,2]. GDM predisposes to future risk of type-2 DM in both the mother and her offspring[3]. Twenty to fifty percent of women with GDM will develop type-2 DM in the 5-10 years after delivery, corresponding to a 7.4-fold increased risk. Untreated GDM during pregnancy may lead to an increased risk of large-for-gestational-age births, low blood sugar, and jaundice in the neonatal period[4,5]. The prevalence of GDM is increasing worldwide proportionate to DM in the population. GDM occurs in up to 14% of all pregnancies in the United States of America (USA), whereas Asians have an 11.3 higher relative-risk of GDM[6]. Indian women with GDM have a higher risk of diabetes and metabolic syndrome[7]. Early detection of glucose intolerance during pregnancy has tri-pronged implications. One, GDM offers a timely opportunity for screening, management, and prevention of GDM and type-2 DM in pregnant women. Secondly, it prevents fetal complications thereby improving neonatal outcomes[8]. Thirdly, it offers the development, testing, and implementation of clinical and epidemiologic strategies for diabetes prevention in the population[9]. In the absence of consensus-based guidelines for the screening and diagnosis of GDM, there are variations in antenatal-care protocols[10]. There are variations between the American Diabetes Association (ADA) recommendations of selective screening vis-a-vis the American College of Obstetricians and Gynecologists (ACOG), which recommends universal screening. Universal mandatory screening for GDM is becoming the standard of antenatal-care even in low-income countries, notwithstanding healthcare equity and accessibility. Most institutions offer a 2-step procedure for screening and diagnosis of GDM as per the Carpenter Coustan criteria (CCC), which is cumbersome and entails additional costs to the exchequer. The international hyperglycemia and pregnancy outcome study results were promulgated by the International Association of Diabetes and Pregnancy Study Groups (IADPSG), which recommended a single-step testing methodology, reducing costs, and improving patient convenience. The IADPSG thresholds of fasting >92 mg/dL, 1 hour ≥180 mg/dL, or 2 hour ≥153 mg/dL plasma venous glucose values after a 75 g oral glucose tolerance test (OGTT) were accepted by the World Health Organization (WHO) and the ADA in 2013 and 2014, respectively, despite having been reported as having lower sensitivity[11-13]. The Diabetes in Pregnancy Study Group of India (DIPSI) criteria are a major breakthrough because they cater for the screening and diagnosis of all pregnant women irrespective of fasting state through a simple, economical, and convenient single-step procedure with a 75 g 2 hour glucose test with a cut-off point of >140 mg/dL for diagnosis. This prospective study was undertaken to validate the single-step non-fasting 75 g DIPSI criteria of GDM in Indian patients in comparison with the two-step fasting 100 g OGTT with the CCC[14].

Materials and Methods

The prospective comparative triple-blind study was conducted with 200 pregnant women who presented to the antenatal clinic of a 1600-bed tertiary-care teaching hospital in Western India over a period of two years from May 2012 to April 2014, after obtaining written informed consents and approval from Armed forces Medical Coleges Ethics Committee. All pregnant women with recorded ≤20 weeks period of gestation (POG) were included. Patients with a history of GDM/impaired glucose tolerance/DM, unexplained stillbirth, a macrosomic baby, congenital anomalies or birth injuries were excluded. Triple-blinding of patients, gynecologists, and pathologists was ensured to eliminate confounding and bias. All 200 patients were subjected to comparative testing through a non-fasting 75 g oral glucose (DIPSI) and fasting 100 g OGTT interpreted by CCC at less than 20 weeks POG and again between 24-28 weeks POG, with a temporal separation of ≤4 days between the non-fasting OGTT with the DIPSI criteria and fasting OGTT with CCC. Plasma venous blood glucose levels were estimated using the hexokinase method on an autoanalyzer (Siemens Healthcare Diagnostics, Inc., West Sacramento, CA 95691 USA). Values ≥140 mg/dL at 2 hours were considered positive with the DIPSI criteria. Any two values from ≥95 mg/dL for fasting, ≥180 mg/dL at 1 hour, ≥155 mg/dL at 2 hours, and ≥140 mg/dL at 3 hours were considered positive with the 100 g OGTT with the CCC for the diagnosis of GDM. Quality control was ensured using internal quality control kits, Levey-Jennings charts based on lab-derived mean and standard deviation, corrective action on violations of Westgard rules, and subscribed external quality controls.

Statistical Analysis

Data were analyzed using SPSS (version 21; IBM Corporation). The patients' clinicodemographic profiles and blood glucose levels were correlated for descriptive statistics including frequency, percentages, and 95% confidence intervals (CI).

Results

The study had a 100% follow-up with no drop-outs. The mean age and body mass index (BMI) of the patients were 24.26±3.75 years and 20.7±3.07 kg/m^2. Of the 200 women, 31/200 (15.5%, 95% CI: 10.93-21.44) tested positive with the DIPSI criteria, and 26/200 (10.5%, 95% CI: 6.77-15.81) tested positive in the 100 gm OGTT as per the CCC. The 169 women who initially tested negative with the DIPSI criteria continued to be negative

on repeat testing with the DIPSI and CCC at 24-28 weeks POG. The prevalence of GDM in the study cohort was found as 15.5% using DIPSI criteria, and the prevalence of GDM after 100 gm OGTT with the CCC was 13% (Table 1). The sensitivity, specificity, and positive and negative predictive values of the DIPSI guidelines were found as 100%, 97.14%, 83.87%, and 100%, respectively. The positive and negative likelihood ratios were 35.8 and zero. Diagnostic accuracy was found as 97.56% (Table 2).

Discussion

The effectiveness of glucose-challenge tests in the non-fasting state for screening and diagnosing GDM has long been a matter of debate. The ADA recommends only selective screening for GDM. Selective screening by risk factors such as woman's age, ethnicity, and BMI may miss some patients with GDM in the lower risk category, whereas more such patients may be diagnosed in the higher risk category. The reason for universal screening for GDM is to try and reduce the number of pregnant women undergoing OGTTs. A universal screening protocol requires the consideration of patient comfort, cost, and the risk of missing the diagnosis. The current ACOG recommendation of universal screening is a more practical approach but it advocates universal screening using two-step methods. Currently, the most used screening test is the oral glucose challenge test (OGCT) with 50 g of glucose followed by an OGTT with 100 g of glucose. Other screening tests and cut-off values are fasting blood glucose (126 mg/dL, 7.0 mmol/L) and random blood glucose (200 mg/dL, 11.1 mmol/L). The diagnostic test for GDM has always been the 100 g 3 hour OGTT. The WHO-IADPSG 75 g OGTT is currently recommended by the WHO for the diagnosis of GDM and it is widely used in Europe. The 100-gm OGTT is still predominantly used in the USA. However, in countries such as Saudi Arabia, Nigeria, and China, a 1 hour 50 g OGCT at 24-28 weeks of gestation is considered as a reliable universal screening test for GDM. Measurements of blood glucose levels in capillary bloods using a glucometer has made screening easy and simple because it can be performed in an office setting and does not require elaborate laboratory facilities, which may be far and few in resource-limited healthcare environments. It is important to know that capillary blood glucose levels are comparable to venous blood glucose levels during the fasting state but are higher after meals[15]. Most institutions offer a 2-step procedure for screening and diagnosis of GDM, under ADA, ACOG, WHO-IADPSG, Canadian Diabetes Association, National Diabetes Data Group (NDDG), National Institute of Health and Care Excellence in the United Kingdom and/or Australasian criteria. Reproducibility has been reported as 78% at best. In the 4th International Workshop Conference on GDM in 1997, a consensus was reached on replacing NDDG criteria with CCC criteria, which have lower threshold values for the diagnosis of GDM[16-18]. The screening and diagnosis of GDM has been simplified from the two-step fasting OGTT under ADA criteria/CCC to single-step fasting OGTT under WHO-IADPSG criteria. The 75 g DIPSI criteria with a 2 hour cut-off value of ≥140 mg/dL is a notch simpler than the WHO-IADPSG criteria because it offers both screening and diagnosis with a single-step non-fasting OGTT, which is immensely practical, economical, feasible, and convenient for patients and obstetric healthcare providers. The DIPSI criteria offer a promising technique with a high sensitivity of 100%, specificity of 97.21%, accuracy of 97.56%, and negative predictive value of 100%, compared with the fasting 100 g OGTT as per the CCC as seen in this study. Various studies have shown higher sensitivity and specificity of non-fasting 75 g two hour DIPSI testing compared with other criteria[19]. The DIPSI criteria have demonstrated 100% sensitivity, 100% specificity, and 94% diagnostic accuracy[20-23].

Table 1. Prevalence of gestational diabetes mellitus (n=200)

GDM	Number of patients	Percentage (%)	95% Confidence intervals
Non-fasting 75 gm Diabetes in Pregnancy Study Group criteria			
Present	31	15.5	10.93-21.44%
Absent	169	84.5	78.56-89.07%
Fasting 100 gm OGTT Carpenter Coustan criteria			
Present	26	13	8.82-18.65%
Absent	174	87	81.35-91.18%

OGTT: Oral glucose tolerance test, GDM: Gestational diabetes mellitus

Table 2. Evaluation of non-fasting 75 gm Diabetes in Pregnancy Study group criteria (n=200)

75 mg DIPSI	100 mg OGTT (Carpenter Coustan criteria)		Total
	Positive	Negative	
Positive	26 (a)	5 (b)	31
Negative	0 (c)	169 (d)	169
Total	26	174	200
Diagnostic indicators			95% CI
Sensitivity		100%	83.98-100%
Specificity		97.21%	93.06-98.93%
Positive predictive value		83.87%	65.53-93.9%
Negative predictive value		100%	97.23-100%
Accuracy		97.56%	-
Positive likelihood ratio		35.8	14.67-82.55%
Negative likelihood ratio		0	2.29-11.77%
Pre-test odds positive		0.14	-
Post-test odds positive		5.2	-

OGTT: Oral glucose tolerance test, DIPSI: Diabetes in Pregnancy Study Group of India
CI: Confidence intervals

Non-fasting OGTT causes the least disturbance to a pregnant woman's routine activities. Even if the DIPSI test is to be repeated in each trimester, the cost of performing DIPSI procedures will be less than the cost of performing any other diagnostic procedures because it requires little preparation, without requiring the prior interposition of the screening test. DIPSI has been proven to be a suitable test with higher sensitivity than WHO-IADPSG criteria in consonance with this study[24,25]. The DIPSI criteria have limitations in comparison with other OGTT criteria as seen in different patient populations, which may be a doubled-edged decision conundrum. The DIPSI criteria may not be able to account for fasting hyperglycemia. False-positive GDM with the DIPSI criteria in the absence of confirmatory GDM tested by other OGTT with low PPV, can lead to psychological stress, clinico-ultrasonographic surveillance, and interventions. False-negative GDM with DIPSI may be labeled as normal and may impact fetomaternal outcomes. DIPSI is based on the observations of the diabetes in pregnancy and awareness project. OGTT irrespective of last meal timing to diagnose GDM has been proven, which is in accordance to DIPSI guidelines. It is important to accept that no test is 100% sensitive or specific or has a 100% PPV and NPV. The WHO-IADPSG criteria have been reported to have lower sensitivity. Certain studies reported a lower sensitivity of DIPSI in comparison with the 75 g OGTT; however, almost all of these studies also reported high specificity and negative predictive values of DIPSI[26-28]. The high negative predictive value with a 75 g non-fasting DIPSI can definitely rule out GDM, thus making DIPSI a convenient and cost-effective screening tool for outpatients in antenatal centers[29-31]. However, the approach has limitations and cannot be concluded as superior to the universal approach with this study. Indian studies reported the prevalence of GDM as between 16.55% and 22% using the DIPSI criteria, which is comparable to the prevalence of 15.5% in this study[32,33]. Challenges in laboratory quality control exist in developing countries conducting mass-screening in resource limited facilities, which affects clinical decision- making[34]. The DIPSI criteria have been included in the guidelines of the Ministry of Health and Family Welfare, Government of India[35-37].

Study Limitations

The study is limited by sample size and unaccounted fasting hyperglycemia.

Conclusion

The DIPSI criteria have high sensitivity, specificity, negative predictive values and diagnostic accuracy. DIPSI offers simplicity, feasibility, convenience, and repeatability, while economizing universal screening and diagnoses of GDM on a mass-scale. The DIPSI procedures have the potential to be applied to the entire obstetric population, in the implementation of public health programs to diagnose GDM in the community, thus reaching the needs of the developing world.

Ethics

Ethics Committee Approval: Approval of Institutional Armed Forces Medical College, Pune Ethics committee taken approval number: AFMC/2011-14/OBG/01.

Informed Consent: Written and informed consent was duly taken by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.K., Concept: S.K., H.B., Design: S.K., D.P., Data Collection or Processing: S.K., D.P., Analysis or Interpretation: S.K., H.B., I.D.K., Literature Search: S.K., H.B., I.D.K., Writing: S.K., I.D.K.

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

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

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.48091

Turk J Obstet Gynecol 2018;15:80-6

Visfatin as a predictor for growth of fetus and infant

Fetüs ve bebeğin büyümesi için bir ön gördürücü olarak visfatin

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Abstract

Objective: Visfatin is an adipocytokine that functions as an enzyme and a growth factor to investigate the relationship between serum visfatin and the fetus's anthropometric markers up to a year after birth.
Materials and Methods: Forty-one eligible pregnant women in their first trimester were divided and matched in terms of body mass index (BMI) before pregnancy into normal and higher than normal BMI groups, A and B. Serum visfatin levels were measured during 6-12 and 15-20 weeks of gestation using ELISA.
Results: The infants were followed up for a mean duration of 10.19±2.83 months. In group A, there was a strong positive relationship between birth head circumference and the first (p_1=0.054, r_1=0.580) and second trimester visfatin levels (p_2=0.051, r_2=0.530). In group B, second trimester visfatin levels correlated negatively with birth length (p=0.015, r=-0.523) and infant's head circumference (p_2=0.050, r_2=-0.392). In a separate study on group B, visfatin levels in the first and second trimesters showed a significant negative correlation with infant's weight. A significant correlation was observed between the first and second trimesters visfatin level with infant's height in both groups, such that this relationship was positive in group A and negative in group B. Linear regression analysis revealed that first and second trimester visfatin levels were significant independent predictors of infant's weight in group B and infant's height in both groups. Second trimester visfatin level was a significant predictor of birth height in group B.
Conclusion: Maternal serum visfatin level shows a relationship with fetal and infant anthropometric indicators, with different effects in the two groups, suggesting visfatin dysfunction in the overweight group before pregnancy.
Keywords: Visfatin, predictor, grow, fetus, infant

Öz

Giriş: Visfatin, sadece enzim olarak değil aynı zamanda bir büyüme faktörü olarak da işlev gören bir adipositokindir. Bu çalışma, serum visfatin ile fetüsün antropometrik belirteçleri arasındaki ilişkinin doğumdan sonraki bir yıla kadar araştırılması amacıyla yürütülmüştür.
Gereç ve Yöntemler: Birinci trimesterdeki 41 uygun gebe kadın, beden kitle indeksi (BKİ) açısından normal ve normal BKİ'den daha yüksek olmak üzere grup A ve B olarak ayrıldı ve eşleştirildi. Serum visfatin düzeyi gebeliğin 6-12 ve 15-20 haftalarında ELISA yöntemi kullanılarak ölçüldü.
Bulgular: Çocuklar, ortalama 10,19±2,83 ay süresince takip edildi. A grubunda, doğum başı çevresi ile birinci (p_1=0,054, r_1=0,580) ve ikinci trimester visfatin düzeyleri (p_2=0,051, r_2=0,530) arasında neredeyse pozitif anlamlı bir ilişki gözlendi. B grubunda, ikinci trimester visfatin düzeyi, doğum boyu (p=0,015, r=-0,523) ve bebeğin baş çevresi [neredeyse anlamlı (p_2=0,050, r_2=-0,392)] ile negatif ilişkilendirildi. B grubu üzerinde yapılan ayrı bir çalışmada, birinci ve ikinci trimester visfatin düzeyleri bebeğin ağırlığı ile negatif anlamlı bir korelasyon gösterdi. Her iki grupta da bebeğin boyu ile birinci ve ikinci trimester visfatin düzeyi arasında anlamlı bir korelasyon gözlendi, öyle ki bu ilişki A grubunda pozitif ve B grubunda negatif idi. Doğrusal regresyon analizi; birinci ve ikinci trimesterdeki visfatin düzeylerinin, B grubundaki bebek ağırlığının ve her iki gruptaki bebek boylarının anlamlı bağımsız ön gördürücü olduğunu ortaya çıkarmıştır. İkinci trimester visfatin düzeyi, B grubundaki doğum boyunun anlamlı bir ön gördürücüsüydü.
Sonuç: Annenin serum visfatin düzeyi, fetüs ve çocuğun antropometrik belirteçleri ile iki grupta farklı etkilerle, gebelik öncesi fazla kilolu grupta visfatin disfonksiyonunu düşündüren bir ilişki göstermektedir.
Anahtar Kelimeler: Visfatin, ön gördürücü, büyüme, fetüs, bebek

PRECIS: We measured the serum visfatin level in pregnancy, and assess its relationship with the children's anthropometric markers, to determine if it can be used as biomarker.

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Received/Geliş Tarihi: 23.12.2017 **Accepted/Kabul Tarihi:** 02.04.2018

Introduction

By releasing adipokines, adipose tissue has a major role in fertility, and physical and sexual maturation[1]. Visfatin, a new 52KDa adipokine[2], was renamed to nicotinamide phosphoribosyl transferase (Nampt) in 2002 after it was shown that it encodes an enzyme called Nampt, which is involved in the conversion of nicotinamide into nicotinamide adenine dinucleotide (NAD)[3]. Intercellular Nampt is substantially released by embryonic, amniotic, and placental membrane and adipose tissue. In a term fetus, amnion and decida have higher levels of mRNA of the visfatin gene[4]. Yet, visfatin's regulatory and release mechanisms in the fetus and neonate are still unclear[5]. It is probably regulated by glucose and insulin[6], and increases with gradual degradation of B-cell and progress of insulin-resistance and maternal weight[7]. Many studies have shown that increased visfatin levels in maternal plasma are associated with small-for-gestantional-age births[8] and intrauterine growth restriction[9]. Serum visfatin levels fluctuate as pregnancy advances[10]. Strong relationships have recently been reported between serum visfatin levels in the first trimester of pregnancy and insulin secretion in the fetus and final birth weight, which shows the role of visfatin secretion in early pregnancy in the later metabolic modeling of the fetus[11]. These results suggest that visfatin may play an important role in maternal-fetal metabolic interaction. However, due to the lack of sufficient information about the physiologic role of visfatin in adults, its source and regulation mechanism in fetal and neonatal stages cannot be argued absolutely. To investigate the role of visfatin in the growth of the fetus and up to a year after birth, we measured serum visfatin levels during 6-12 and 15-20 weeks of gestation in an Iranian population.

Materials and Methods

The present cohort study was conducted between 2013 and 2015 after obtaining approval of the Research Council of Tarbiat Modares University, Tehran, Iran, and permission from the Medical Ethics Committee of the Faculty of Medical Sciences, and presenting a letter of introduction from the university to selected and densely populated medical centers covered by Shahid Beheshti University of Medical Sciences in the north, east, and Shemiranat districts of Tehran, Iran. This study was approved by the Ethics Committee of Tarbiat Modares University (registration number: IR.TMU.REC.1394.120). The study is registered in the Iranian Research Institute for Information Sciences and Technology. A flow chart of the study is presented in Figure 1. First, pregnant women were briefed on the study objectives and the confidentiality of maternal and neonatal information, and they then signed informed consents. Then, 41 eligible pregnant women in their first trimester were selected through convenience sampling. The study inclusion criteria were age 18-40 years, singleton pregnancy, no systemic diseases such as lupus and diabetes mellitus, and Iranian nationality. The study exclusion criteria were pregnancy complications

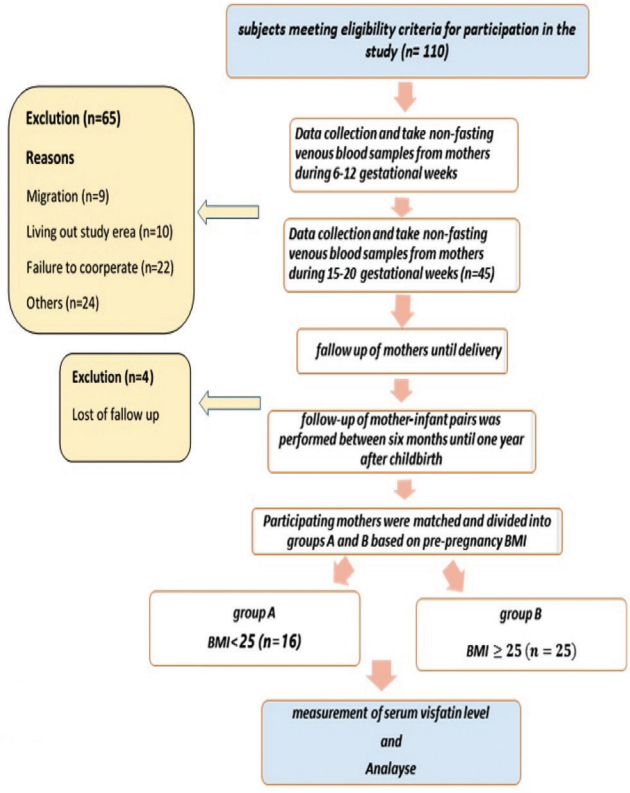

Figure 1. Flow chart of the cohort
BMI: Body mass index

(e.g., diabetes, preeclampsia), psychological problems, tobacco or alcohol use in the first trimester of pregnancy, medications other than pregnancy supplements, abnormal stresses such as family bereavement and accidents, migration, living outside of the study area, failure to cooperate, follow-up exitus, and becoming pregnant again within a year. Demographic details, pregnancy history and maternal medical history were found through direct interviews with mothers in their first pregnancy visit, based on the ministry of health's routine prenatal care questionnaire. Gestational age was calculated based on the first day of the last menstruation (LMP), or the first trimester ultrasound (if uncertain about LMP). Weight, blood pressure, and heart rate of the fetus were measured by the same person using a digital scale, digital barometer, and fetal heart detector (sonicaid). At the end of their visit, mothers received training on proper pregnancy nutrition. The researcher took non-fasting venous blood samples from mothers during 6-12 and 15-20 gestational weeks between 09:00 and 11:00 am. The samples were added to ethylenediaminetetraacetic acid-containing tubes and sent to endocrinology and metabolism laboratories of Shahid Beheshti University of Medical Sciences, Tehran within 24 hours and kept at 2-8 °C. For plasma separation, samples were centrifuged at 3000 rpm and 4 °C for 10 minutes. The resulting plasma was frozen at a temperature of ≤-20 °C until required for analysis. Anthropometric indicators (birth height, weight, and head circumference) were measured. The next

follow-up of infants was performed between six months and one year after childbirth (both cesarean and vaginal) through telephone contact with the mothers, during which they were asked to attend their local health centers for the assessment of anthropometric indicators (growth monitoring curve, height, weight, and head circumference) and also neonatal diseases. Children were assessed based on the ministry of health's questionnaire for children under 8 years of age. Afterwards, participating mothers were matched and divided into groups A and B based on pre-pregnancy body mass index (BMI) as follows: normal (18.5-25 kg/m^2) (n=16) and overweight and obese (≥25 kg/m^2) (n=25). Pre-pregnancy BMI based on pre-pregnancy weight was calculated using the following equation as defined by the World Health Organization: weight/height^2. BMI between 18.5 and 25 kg/m^2 is considered normal, and ≥25 kg/m^2 is abnormal.

Statistical Analysis

The homogeneity of the two groups was confirmed through statistical tests in terms of underlying and demographic variables such as age, education, employment, household size, and neonatal sex, fertility status, income, childbirth method, and family planning, the time presenting for postpartum childcare, feeding method (formula, breast milk, or both), hypothyroidism, neonatal anemia, and referral to physician. Serum visfatin levels were measured using enzyme-linked immunosorbent assay (ELISA) with human visfatin (ZellBio GmbH, Germany; Ulm kit, Cat No: ZB-3408-H9648). Finally, visfatin levels were compared using data from maternal and neonatal anthropometric measurements. Normal distribution of variables in each group was assessed using the Kolmogorov-Smirnov test. The independent and paired t-test were used for normally distributed quantitative variables, the chi-square test was used for qualitative variables, and the Mann-Whitney U and Wilcoxon Signed-Rank tests were used for non-normally distributed variables. The relationships among the present study variables in each group were separately assessed using independent t, Pearson's, linear regression, and Spearman's ordinal correlation tests. Finally, lambda stat was used to compare correlation coefficients of variables in the two groups at $p<0.05$.

Results

Demographic and underlying variables from the two groups are presented in Table 1.

Variations in visfatin level during pregnancy

Mean non-fasting serum visfatin level of participating mothers (n=41) was 59.4±68.01 ng/mL (range, 4.9-234 ng/mL) in the first trimester, and 76.98±75.55 ng/mL (range, 4.6-248 ng/mL) in the second trimester, and variations in visfatin level were reported as 17.58±26.46 ng/mL.

The relationship between visfatin level and neonatal anthropometric indicators at birth

In group A, a strong positive relationship was observed between birth head circumference and the first (p_1=0.05, r_1=0.580) and second trimester visfatin levels (p_2=0.051, r_2=0.530). Spearman Rank correlation test showed a negative significant relationship between second trimester visfatin level and birth height (p=0.015, r=-0.523) in group B (Table 2). Linear regression analysis revealed that the second trimester visfatin level was a significant predictor of birth height in group B (Table 3).

The relationship between visfatin level and infant's anthropometric indicators

The mean duration of neonatal follow-up was 10.19±2.83 months. Significant correlations were observed in both groups between first trimester visfatin (p_a=0.002, r_a=0.713, p_b=0.005, r_b=-0.540, in A and B group, respectively) and second trimester visfatin (p_a=0.009, r_a=0.628 and p_b=0.008, r_b=-0.518, in groups A and B, respectively) with infant's height. This correlation was positive in group A and negative in group B. According to the Lambda test, this correlation was significantly greater in group A than in group B ($p<0.05$). A negative and significant correlation was separately found between infant's weight and the first (p_1=0.024, r_1=-0.450) and second trimester visfatin levels (p_2=0.005, r_2=-0.540) in group B. Generally, the relationship between visfatin and the infants's growth indicators was positive in group A and negative in group B. A strong negative correlation was separately found between the second trimester visfatin level and infant's head circumference (p_2=0.05, r_2=-0.392) in group B (Table 2). Linear regression analysis revealed that first and second trimester visfatin levels were significant predictors of infant's weight in group B and infant's height in both groups (Table 3).

Discussion

No study has yet been conducted to assess the relationship between maternal serum visfatin and neonatal anthropometric indicators by categorizing mothers based on BMI. The visfatin regulation mechanism in human fetal blood circulation is unknown[12]. However, recent studies have proposed the presence of a relationship between maternal serum visfatin level and fetal growth. A strong relationship was found between maternal serum visfatin and neonatal anthropometric indicators in groups A and B, with a possible mechanism of maternal visfatin entering the fetal blood circulation through the placenta as a result of endocrine changes. In a study by Cekmez et al.[13], anthropometric indicators, including weight and height, were measured and plasma lipids, insulin, and adiponectin and visfatin concentrations in the umbilical cord blood of 50 large-for-gestational age (LGA) and 50 appropriate-for-gestational age (AGA) infants born following complication-free pregnancies were assessed. The mean visfatin and adiponectin levels were significantly higher in the macrosomia group compared with the AGA group. Moreover, umbilical cord serum visfatin concentrations were found to have a positive relationship with insulin levels[13]. Mazaki-Tovi analyzed the relationship of increased maternal serum visfatin with gestational diabetes mellitus infection and the birth of LGA infants. An increase in maternal blood glucose leads to a rise in fetal blood glucose, which in turn stimulates fetal pancreatic islet cells, resulting

in hyperinsulinemia. In LGA infants, hyperinsulinemia in fetal period leads to fetal macrosomia[14]. One of the reasons for a visfatin effect on fetal development is its effect on sirtuins (SIRT). SIRTs are a class of proteins that have a role in cellular processes associated with body metabolism such as cell differentiation, aging, transcription, apoptosis, inflammation, and stress resistance, as well as energy efficiency and alertness during low-calorie situations. Humans have seven SIRT isoforms. SIRT 1 (SIRT1) affects chromatin modulation, and therefore suppresses transcription and interacts with transcription factors, and is capable of positive or negative regulation of gene expression[15]. Through involvement in NAD^+ synthesis needed for SIRT activity, visfatin has a major role in the regulation of SIRT1-dependent transcription, and this leads to the metabolism of energy and differentiation of stem cells. SIRT Nampt expression increased in both cell models in the course of bone tissue differentiation from multipotent fibroblast and monopotent pre-osteoblast in rats. A rise in Nampt leads to higher concentrations of NAD^+ and higher activity of SIRT1. In contrast, a reduction or inhibition of Nampt leads to reduced NAD^+ concentration and SIRT1 activity, resulting in inhibition of osteocyte differentiation. This means that Nampt promotes osteocyte differentiation through a pathway mediated by SIRT1[16,17]. High levels of SIRT1 expression in the human brain have been revealed[18]. The early expansion of neurons initiates with neurite process elongation pursued by axon differentiation, dendritic arborization, and synapse formation. SIRTs have an important role in the process of synapse promotion and modulation of their strength, which is important for memory formation. During neuronal development, SIRT1 has a important role in promoting xonal elongation, neurite outgrowth, and dendritic

Table 1. Demographic and clinical characteristics of pregnant women (n=41)

Characteristics		Group A (n=16) n (%)	Group B (n=25) n (%)	p value
Mother's education	5 years	2 (12.5)	6 (24.0)	0.11
	8 years	2 (12.5)	6 (24.0)	
	12 years	9 (56.2)	13 (52.0)	
	More than 12 years (university)	3 (18.8)	0 (0)	
Husband's education	Illiterate	0 (0)	1 (4)	0.68
	5 years	1 (6.3)	3 (12)	
	8 years	8 (50)	9 (36)	
	12 years	6 (37.5)	8 (32)	
	More than 12 years (university)	1 (6.3)	4 (16)	
Mother's job	Housewife	16 (100)	23 (92.0)	0.20
	Employee	0 (0)	2 (8.0)	
Husband's job	Self-employed	14 (87.5)	20 (80.0)	0.53
	Employee	2 (12.5)	5 (20.0)	
Family income	Less than 1.000.000 rials	3 (18.7)	2 (8.0)	0.56
	Between 1.000.000 and 3.000.000 rials	10 (62.5)	19 (76.0)	
	More than 3.000.000 rials	3 (18.8)	4 (16.0)	
Baby sex	Male	6 (37.5)	12 (48.0)	0.54
	Female	10 (62.5)	13 (52.0)	
Type of delivery	NVD	7 (43.7)	14 (56.0)	0.98
	CS	9 (56.3)	11 (44.0)	
Parity	Nulliparous	8 (50)	6 (24.0)	0.09
	Multiparous	8 (50)	19 (76.0)	
First 6 months of lactation	Exclusive	14 (87.5)	12 (48)	0.056
	Dominant	2 (12.5)	6 (24)	
	Breastfeeding + formula feeding	0 (0)	5 (20)	
	Formula feeding	0 (0)	2 (8)	

NVD: Normal vaginal delivery, CS: Cesarian section

Table 2. The relationship between visfatin level and anthropometric indicators

Serum visfatin level (ng/mL) / Anthropometric indicators (ng/mL)	Spearman rank correlation test (n=16) Group A		(n=25) Group B	
	First trimester serum visfatin level	Second trimester serum visfatin level	First trimester serum visfatin level	Second trimester serum visfatin level
Infant's height	r=0.713 p=0.002	r=0.628 p=0.009	r=-0.540 p=0.005	**r=-0.518** **p=0.008**
Infant's weight	r=0.393 p=0.132	r=0.394 p=0.132	r=-0.450 p=0.024	**r=-0.540** **p=0.005**
Infant's head Circumference	r=0.417 p=0.108	r=0.272 p=0.309	r=-0.375 p=0.065	r=-0.392 p=0.050
Birth height	r=0.041 p=0.889	r=0.029 p=0.922	r=-0.384 p=0.085	r=-0.523 p=0.015
Birth head Circumference	r=0.580 p=0.054	r=0.530 p=0.051	r=0.047 p=0.847	r=-0.002 p=0.994

Table 3. Lenear regression analysis between anthropometric indicators as dependent variable and serum visfatin level

Dependent variables				Anthropometric indicators		
Independent variable Infant's height				Birth height	Infant's height	Infant's weight
Serum visfatin level	**Group A (n=16)**	First trimester serum visfatin level	β	NS*	0.06	NS
			p value		0.002	
		Second trimester serum visfatin level	β	NS	0.05	NS
			p value		0.009	
	Group B (n=25)	First trimester serum visfatin level	β	NS	-0.03	-9.194
			p value		0.02	0.04
		Second trimester serum visfatin level	β	-0.015	-0.03	-8.27
			p value	0.01	0.008	0.01

*NS: Non-significant, non-significant p values are not reported

branching via several targets and mechanisms[19]. Increasion of nerve growth factor in PC12 cells by Cytoplasmic SIRT1 Induced neuritogenesis[18]. The NAD^+ dependent deacetylase SIRT1 is implicated in energy balance regulation by its effect on pro-opiomelanocortin and agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamusthis[20]. Reduction of energy intake induced SIRT1 in brain[21]. Obesity is correlated with low NAD^+/SIRT pathway expression in adipose tissue of BMI-discordant monozygotic twins[22]. The selective knock-out of SIRT1 in hypothalamic AgRP neurons diminished response to hunger-inducing hormone ghrelin, reduced food intake, consequently causing decreased lean mass and body weight[23]. Visfatin is expressed in bovine mammary epithelial cells, lactating mammary gland, and milk, which is regulated by the cAMP pathway[24]. The persistence of the relationship between postpartum maternal serum visfatin levels and anthropometric indicators can be attributed to excretion of visfatin in mother's milk, which was confirmed by Bienertová-Vašků et al.[25]. They examined milk and venous blood samples of 24 healthy breastfeeding women with complication-free physiologic pregnancies and neonates with appropriate weight for gestational age at birth and 180 days after birth. Visfatin had been copiously excreted in mother's milk, and serum visfatin levels were 100 times higher in mothers' milk than in their blood. Significant changes occurred in maternal serum visfatin levels after childbirth. Based on maternal colostrum visfatin levels, the aurors were able to predict neonatal weight loss in the first three days of birth[25,26]. Visfatin by effect on the production

of SIRT1, consequently, interference in the development of the brain and energy balance regulation may be associated with head circumferences and weights of fetus and child. According to these results, visfatin can have a major role in regulating neonatal obesity after birth. The above studies confirm the present study results. Generally, these results show that visfatin has a major role in maternal-fetal metabolic interaction. Our results further show different metabolic regulations in groups A and B, which may be due to differences in expression of SIRT1, glucose transfer or insulin resistance as a result of resistance to glucose entry to fetal tissues for unknown reasons, which require further studies. It is likely that visfatin can be used in the future as a biomarker predicting fetal and neonatal growth.

Study Limitations

This study provides useful data of the relationship between serum visfatin and the children's anthropometric markers up to a year after birth. However, the present study has some limitations. First, we know that it would be better to evaluate levels of cord plasma and breast milk concentrations of visfatin and assess their correlation with the children's anthropometric in order to support our results. However, we could not do it, which is a limitation of our study. Second, the fallow up of mothers and infants was short-term. We suggest the long-term fallow up of mothers and infants with more abundant sample.

Conclusion

After a careful review of previous studies on visfatin and pregnancy, we can claim the present study is the first in Iran to have specifically addressed the relationship between maternal visfatin levels and infant's anthropometric indicators up to a year after birth. Our study can be the basis for further and more precise studies. According to the present study results, mean serum visfatin levels were higher in the second trimester compared with the first. The mean maternal serum visfatin level during pregnancy significantly increased with increasing gestational age in parallel to weight gain and insulin resistance in both groups separately. In the present study, the relationship between prenatal visfatin level and infant's anthropometric indicators in the two years following childbirth was assessed. Serum visfatin level appears to be related to the fetus and infant's anthropometric indicators (infant's weight, height and birth height. First and second trimester visfatin levels were significant independent predictors of infant's weight in group B and infant's height in both groups. The second trimester visfatin level was a significant predictor of birth height in group B. Visfatin has extensive effects on pregnancy physiology and pathology, maternal and especially neonatal outcomes, and its effect is different in normal weight and overweight women. Hence, pre-pregnancy BMI appears to be a determining factor in creating the difference and the amount of maternal plasma visfatin during pregnancy, which can predict maternal serum visfatin and neonatal and maternal outcomes. It is recommended that future studies be conducted with larger sample sizes and longer follow-up periods.

Acknowledgements

We acknowledge Dr. Mehdi Hedayati and Miss Hoghoghi, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, for conducting the ELISA analysis of Visfatin and midwives of Mahdieh hospital prenatal clinic and health centers in north, east and Shemiranat in Tehran for assisting with the taken non-fasting venous blood samples from mothers and all participating mothers in our research.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Tarbiat Modares University (registration number: IR.TMU.REC.1394.120).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.84429

Turk J Obstet Gynecol 2018;15:87-90

Investigation of the embryotoxic and teratogenic effect of *Hypericum perforatum* in pregnant rats

Hypericum perforatum'un gebe sıçanlarda embriyotoksik ve teratojenik etkisinin araştırılması

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Abstract

Objective: *Hypericum perforatum* (HP) is a herbal product used in the treatment of depression, but its harm on the fetus has not been established. This study investigated the effects of HP according to fetal clinical, morphologic, and histologic findings. Study design is an animal study. **Materials and Methods:** Fifty-four 4-5-month-old female Wistar rats were divided into three groups: control, 100 mg/kg HP, and 300 mg/kg HP. HP treatment using drinking water was started one week before mating and ended with the delivery of pups. **Results:** HP exposure before conception diminished the pregnancy rate and decreased the fetal number; during pregnancy it tended to increase the duration of gestation, and deteriorated the fetal development as determined using body weight. It also damaged liver and kidney tissues, most probably due to oxidative stress, as supported through inducible nitric oxide synthase antibody staining findings at both doses. **Conclusion:** HP should not be recommended to women who would like to be pregnant or are pregnant because it can be harmful for both fetal and maternal health.

Keywords: Pregnancy, rat, St. Johns Worth, teratogenity

Öz

Amaç: *Hypericum perforatum* (HP) depresyon tedavisinde kullanılan bitkisel bir üründür, fakat fetüs üzerindeki zararı henüz tam belirlenememiştir. Bu çalışma fetüsun klinik, morfolojik ve histolojik bulgulara göre HP'nin etkilerini araştırmayı amaçlar. Çalışma tasarımı, hayvan araştırmasıdır. **Gereç ve Yöntem:** Dört-beş aylık dişi 54 Wistar sıçan üç gruba ayrıldı: Kontrol, 100 mg/kg HP ve 300 mg/kg HP grupları. HP tedavisi çiftleştirmeden bir hafta önce başlayıp, yavruların doğumu ile sona erdi; içme suyu ile verildi.
Bulgular: Konsepsiyon öncesi HP maruziyeti gebelik oranını düşürdü ve fetüs sayısını azalttı gebelik sırasında gebelik süresini uzatma eğilimindeydi ve vücut ağırlığından belirlediğimiz üzere fetal gelişimi bozdu. Hatta, indüklenebilir nitrik oksit sentazi antikor boyaması ile bu çalışmamızı desteklediğimiz üzere, her iki dozda da, büyük olasılıkla oksidatif stress nedeniyle karaciğer ve böbrek hasarına yol açtı. **Sonuç:** HP hamile kalmak isteyen ya da hamile olan hiçbir kadına önerilmemelidir, hem anne hem bebek sağlığı üzerinde tehdit edicidir.

Anahtar Kelimeler: Gebelik, sıçan, St. Johns Worth, teratojenite

PRECIS: Considering of required studies about *Hypericum perforatum* (HP) and fetal exposure, this study has been evaluated adverse effects of HP on fetus both with the clinical findings and histopathological assessment of fetal liver and kidney.

Introduction

Hypericum perforatum (HP), of the Clusiaceae (Hypericeae=Guttiferae) family, belongs to the subfamily Hypericoideae[1-4]. HP contains several groups of components that contribute to its pharmacologic activity. These are naphthodianthrones (hypericin, pseudohypercin), phloroglucinols (hyperforin, adipherforin), flavonoids (rutin, hyperoside, quercitrin) xanthones and tannins. HP has recently

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Received/Geliş Tarihi: 06.02.2018 **Accepted/Kabul Tarihi:** 27.03.2018

received interest as a herbal product that has anti-inflammatory and antiviral properties, and is effective for wound healing, inflammatory bowel disease, and depression[1,5]. Depression is an important disease that affects the whole society at all ages and the incidence in pregnancy period is reported as 18-19%[6]. It is known that the antidepressant activity of the plant (at doses of 900 mg/kg) is related to hypericin and its derivatives. It can also be abused for believing well-being and well-feeling (Biggs et. al.[7] 2017). There are no well-established controlled clinical trials evaluating the safety of HP use, even in the European Medical Agency Guidelines (EMA/HMPC/244315/2016, 2018),[8] for patients who want to become pregnant or are pregnant while under HP treatment. The view that "herbal products are less harmful" is misleading; their use without rigorous research can also bring about important health issues. Therefore, we aimed to investigate the embriotoxic/teratogenic effects of HP according to fetal clinical, morphologic, and histologic findings.

Materials and Methods

Fifty-four 4-5-month-old female Wistar rats were obtained from the experimental animal center of our university and all tests were conducted according to the principles and guidelines of the university animal ethical committee's approval (HADYEK 2015/67). HP was obtained from local pharmacy store (St. John's Wort Herb Extract/SOLGAR İstanbul, Turkey). On the study day, the rats were randomly assigned to three groups of 18 animals. Control group: This group of rats has been taken with water freely and served as control.

Low dose HP group: 100 mg/kg HP given to the rats with drinking water, which was available ad libitum.

High dose HP group: 300 mg/kg HP given to the rats with drinking water, which was available ad libitum.

HP treatment was started one week before mating, similar to the Gregoretti et al.[9] study, and continued till delivery. The rats were weighed every Monday to adjust the HP doses. The calculated amount of HP was mixed with drinking water every morning, making sure that there was no remnants from the previous day. The suggested daily dose of HP is 900 mg (or 15 mg/kg per day for a 60-kg person) for humans; Rayburn et al.[10], calculated the rodent dose as 180 mg/kg per day in their study. Gregoretti et al.[9] calculated the surface area of rats and determined the dose as 100 and 1000 mg/kg for rats. Given that our aim was not to work with high doses, rather just to mimic real life, considering Rayburn et al.'s[10] study, we administered two different doses of HP treatment to gain a better understanding of dose effect and decided upon 100 mg/kg and 300 mg/kg.

Maternal rats were sacrificed under general anesthesia of ketamine and xylazine (50 and 5 mg/kg, respectively) immediately after delivery. Obtained offspring were decerebrated, then morphologically examined and fixed in formalin solution. The obtained preparations were evaluated using hematoxylin and eosin and immunohistochemical staining.

Statistical Analysis

All biologic parameters were assessed using the Mann-Whitney U test (IBM SPSS Statistics for Windows; Version 19.0, IBM Corp., NY, US).

Results

Morphologic-clinical assessment

The number of fetuses was six in the low-dose (100 mg/kg) group, three in the high-dose (300 mg/kg) group, and eight in the control group; the pregnancy rate decreased in a dose-dependent manner (Table 1). In the high-dose treatment group there was also a tendency for delayed delivery; more offspring were born on day 22 (Table 1). The total number of pups also decreased (Table 2); the difference was statistically significant (p=0.014). No structural extremity anomalies, facial anomalies or differences of eye openness were observed in any pups. Regarding the weight and length of the fetuses, there was a 19.9% reduction in the weight of the fetuses of the low-dose group, and an 8.4% reduction in the high-dose group (Table 3).

Hematoxylin and eosin assessment

Our histologic evaluation showed an inflammatory reaction in the liver of the offspring of both treated groups. Additionally, focal necrosis was detected in each lobe, deteriorating cell layout at 300 mg/kg. Hydropic and vacuolar degeneration was also observed in the fetuses of rats with high-dose HP treatment. No fatty change of the liver was found. Hematopoiesis was not disrupted and continued in the fetal period.

Table 1. Morphologic evaluations of the control group and treated groups

Delivery day/the number of pregnancy	Day 21	Day 22	Macroscopic anomalies
Control group	4 rats	4 rats	No
100 mg/kg HP group	4 rats	2 rats	No
300 mg/kg HP group	1 rats	2 rats	No

HP: *Hypericum perforatum*

Table 2. Number of pups in all groups

Study groups/the number of pups born	Day 21	Day 22
Control group	1st birth: 19 pups 2nd birth: 10 pups 3rd birth: 12 pups 4th birth: 10 pups	1st birth: 15 pups 2nd birth: 14 pups 3rd birth: 13 pups 4th birth: 10 pups
Low-dose HP group	1st birth: 12 pups 2nd birth: 16 pups 3rd birth: 8 pups 4th birth: 14 pups	1st birth: 12 pups 2nd birth: 12 pups
High-dose HP group	1st birth: 16 pups	1st birth: 11 pups 2nd birth: 11 pups

HP: *Hypericum perforatum*

In the kidney tissues, we found that the diameter of glomeruli was decreased, the Bowman capsule distance was absent and intense congestion was observed equally in the offspring of both plant-treated groups. Additionally, hydropic and hyaline degeneration was seen in kidney tubules (Figure 1).

Immunohistochemical assessment

Inducible nitric oxide synthase (iNOS) antibody staining of the tissues was used to determine oxidative stress parameters. Levels of damage were determined in fetal liver tissues of rats as low (++) and high (+++). When evaluating oxidative damage in kidney tissues of the fetuses, the damage was determined as low (++) and high (+++) (Figure 2).

Table 3. Morphologic evaluations of the offspring in all groups

Study groups/ morphologic parameters	Average length (cm)	Tail length (cm)	Weight (g)
Control group	3.89	1.34	6.59
Low-dose HP group	3.86	1.5	5.28
High-dose HP group	3.86	1.34	6.04

HP: *Hypericum perforatum*

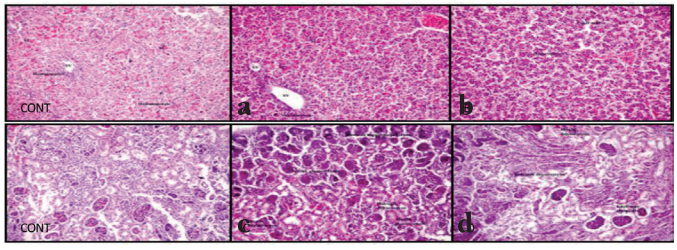

Figure 1. Hematoxylin and eosin (X20) staining of control group and treated individuals. a) Liver tissue of rat embryo treated with low dose 100 mg/kg *Hypericum perforatum* (HP) treatment, b) Liver tissue of rat embryo treated with high-dose 300 mg/kg HP treatment, c) Kidney tissue of rat embryo treated with low dose 100 mg/kg HP treatment, d) Renal tissue of rat embryo treated with high-dose 300 mg/kg HP treatment

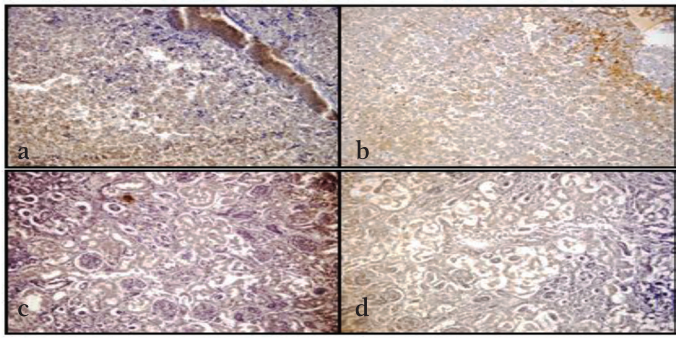

Figure 2. Inducible isoform immunohistochemistry (X20) staining for treated individuals. a) Liver tissue of rat embryo treated with low dose 100 mg/kg *Hypericum perforatum* (HP) treatment, b) Liver tissue of rat embryo treated with high-dose 300 mg/kg HP treatment, c) Kidney tissue of rat embryo treated with low dose 100 mg/kg HP treatment, d) Renal tissue of rat embryo treated with high-dose 300 mg/kg HP treatment

Discussion

As in all herbal medicines, HP is considered innocuous and widely used against depression, and even women who are pregnant or lactating are also exposed to HP[6]. However, the effects of its use on gestation have yet to be clarified[8].

Limited numbers of experimental studies of HP are available; different results have been obtained in animal experiments. In one study, 36 mg/kg/day was given to 15 rats in the organogenesis period (days 9-15), they were sacrificed on the 21st day of pregnancy, and the number of fetuses and resorption rates were calculated during a laparotomy. The size of the fetuses was also measured and the result of the clinical examination showed that HP was not embryo toxic. Given that the fluid and food intake and weight change of the animals were the same as those of the control rats, HP was not found as toxic for the mothers either, but a histologic examination was not included in this study[11]. That study is similar to our work in some direction; no macroscopic difference was determined in the offspring. However, unlike our study, we also found that the rate of conception decreased dose-dependently by giving HP one week before mating. Additionally, the high-dose treatment groups' delivery day tended to delay. Previously, the Calcium channel antagonist properties of HP were shown on rat aorta; in this case, HP might also behave as a dose-dependent tocolytic agent[4]. On the other hand, the conception rate and the number of pups also decreased. This point needs further investigation regarding the ovarian capacity effect of HP. At some point, 100 mg/kg or 300 mg/kg HP was enough to cause a detrimental effect on the gestation rate, gestation duration, and offspring number. Regarding the weight and length of the fetuses, there was a 19.9% and 8.4% reduction in the weight of the fetuses. Only the findings of Rayburn et al.,[12] support our findings; they also found birth weights of male mice were less than controls. Two of Rayburn et al.'s[12] studies were on cognitive-behavioral changes and the authors reported its safety with regards cognitive functions, but a toxicity and histopathologic evaluation on pups tissues were not performed[10]. In a study, HP was started on the 3rd gestational day and ended on the 21st postnatal day. The authors found no effect on the duration of gestation or offspring body weight alteration, but they described some treated groups weighed significantly less than the controls on the 56th postnatal day. As a result, HP was found to affect the development of mice without seriously affecting their neurobehavioral development[13]. Chan et al.[14] concluded that giving the active component, hypericin (14.2 and 142.0 ng/mL), to embryo cultures was teratogenic on rat embryos.

In another study, HP was administered via gavage to rats at two different doses, 100 mg/kg and 1000 mg/kg, starting 2 weeks prior to application and continued till day 21 of lactation[9]. When the mothers took 100 mg/kg HP, hepatocyte cell vacuolization

was determined in the liver of fetuses and 1000 mg/kg treatment increased hepatocellular damage with hyaline degeneration, lobular fibrosis, and disorganization of hepatocytes arrays[9]. Their study supports our histologic findings in a great measure. HP showed an inflammatory reaction in the fetal liver tissue in both treated groups. Additionally, 300 mg developed focal necrosis, and hydropic and vacuolar degeneration of fetal liver tissues. Hematopoiesis was not disrupted and continued in the fetal period, and no fatty change in the liver was seen. The same paper also proved that glomerular size was reduced, the Bowman capsule was absent, and that hyaline tubular degeneration developed. Interestingly, these findings were also found in the offspring, even when they were only exposed to HP during the 21-day lactation period[9]. Similar to that study, we determined that the diameter of glomeruli was decreased, the Bowman capsule distance was absent, and intense congestion was observed equally in the offspring of both plant-treated groups. Additionally, we determined hydropic and hyaline degeneration in kidney tubules. The structural changes that we detected in the liver and kidney were probably due to free oxygen radical generation and consequently to oxidative damage. There are three different NOS enzyme isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and iNOS, which are stimulated by certain cytokines. In pathologic conditions, macrophages and smooth muscle cells in hepatocytes induce iNOS and produce nitric oxide (NO). Excess production of NO results in oxidative tissue damage. Immunohistochemically, we applied iNOS antibody staining to detect the presence of oxidative damage. We demonstrated that the HP produced oxidative damage in the liver and kidney tissues of the fetuses.

Study Limitations

The limitation of this study is HP effects on the other organs such as neuro-development could not been detected, further studies should be performed about safety of HP.

Conclusion

HP exposure before conception diminished the pregnancy rate and decreased the fetal number; during pregnancy it tented to increase the duration of gestation, and deteriorated fetal development as determined through body weight. It also damaged liver and kidney tissues, most probably due to oxidative stress at both doses, as supported with iNOS antibody staining. Therefore, HP should not be recommended to any women who want to be pregnant or who are pregnant, because it can be harmful for both fetal and maternal health.

Ethics

Ethics Committee Approval: Adnan Menderes University for Animal Experiments (HADYEK 2015/67).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: This study was supported by the grant (TPF 15063) of Adnan Menderes University.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.95881

Turk J Obstet Gynecol 2018;15:91-4

Diagnostic value of neutrophil to lymphocyte ratio in differentiation of ruptured ovarian cysts and adnexal torsion

Nötrofil lenfosit oranının rüptüre over kisti ve adneksiyel torsiyon ayrımındaki tanısal değeri

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Abstract

Objective: Ovarian cyst rupture and adnexal torsion (AT) differential diagnosis is important for early surgical intervention of AT for preserving ovarian function. The aim of this study was to evaluate the diagnostic value of preoperative the neutrophil-to-lymphocyte ratio (NLR) in patients with adnexal torsion and ovarian cyst rupture.
Materials and Methods: Data of 80 patients who underwent surgery between 2012 and 2017 for ovarian cyst rupture, adnexal torsion, and unruptured ovarian cyst were analyzed. Patients were categorized as adnexal torsion (n=35), ovarian cyst rupture (n=20), unruptured ovarian cyst (n=25) groups. Preoperative NLR were compared among the three groups of the patients.
Results: The adnexal torsion group had a median NLR of 8.0 (range, 4.0-14.1), the ovarian cyst rupture group had a median of NLR 7.5 (range, 3.7-11.5), and median NLR of the unruptured ovarian cyst group was 2.2 (range,1.8-2.7). The NLR was found to have a difference that reached statistical significance among the three groups (p<0.001). When the groups were individually compared, there was no significant difference between the ovarian cyst rupture and adnexal torsion groups (p=0.372), but there was a significant difference between the unruptured ovarian cyst and adnexal torsion groups (p<0.001).
Conclusion: NLR may be useful in the differential diagnosis of unruptured ovarian cyst from adnexal torsion, but it has no diagnostic value for the differentiation of ovarian cyst rupture and adnexal torsion.
Keywords: Adnexal torsion, ovarian cyst, rupture, neutrophil, lymphocyte

Öz

Amaç: Over kist rüptürü ile adneksiyel torsiyonun erken ayırıcı tanısı over fonksiyonunun korunabilmesi için gerekli acil cerrahi müdahalenin yapılabilmesi için önemlidir. Bu çalışmada nötrofil lenfosit oranının adneks torsiyonu ve over kist rüptürü ayrımındaki tanısal değerinin araştırılması hedeflenmiştir.
Gereç ve Yöntemler: 2012 ile 2017 yılları arasında over kisti rüptürü (20), rüptür olmamış over kisti (25) ve adneks torsiyonu (35) nedeniyle opere olmuş 80 hastanın verileri analiz edildi. Preoperative nötrofil-lenfosit oranları üç grup arasında karşılaştırıldı.
Bulgular: NLR ortanca değerleri, adneksiyel torsion grubu için 8,0 (4,0-14,1), over kist rüptür grubu için 7,5 (3,7-11,5) ve rüptüre olmamış over kist grubu için 2,2 (1,8-2,7) olarak bulunmuştur. Üç grup için NLR arasındaki fark istatistiksel öneme ulaşmıştır (p=0,00). Gruplar bireysel olarak analiz edildiğinde over kist rüptürü ve adneks torsyonu arasında NLR açısından istatistiksel olarak anlamlı fark saptanmazken (p=0,372), rüptüre olmamış over kistleri ile adneksiyel torsiyon grupları arasında istatistiksel olarak anlamlı fark saptanmıştır (p=0,00).
Tartışma: NLR adneksiyel torsiyonun rüptüre olmamış over kistlerinden ayrımında kullanılabilir ancak rüptüre olmuş over kistlerinin ayrımında kullanışlı değildir.
Anahtar Kelimeler: Adneks torsiyonu, over kisti, rüptür, nötrofil, lenfosit

PRECIS: Neutrophil to lymphocyte ratio can be used in preoperative differential diagnosis of ovarian cysts and adnexal torsion. But the diagnostic value of Neutrophil to lymphocyte ratio in differentiation of ruptured ovarian cyst from adnexal torsion cases was not determined in this study.

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Received/Geliş Tarihi: 05.04.2018 **Accepted/Kabul Tarihi:** 26.04.2018

Introduction

Adnexal torsion (AT) can be defined as total or partial rotation of the adnexa around its own vascular axis. It results in venous and lymphatic blockage of ovarian tissue and causes congestion and hemorrhagic infarction leading to gangrene and hemorrhagic necrosis[1]. AT cases are generally seen in the reproductive age group[2]. Although AT has the highest incidence among women aged between 20 and 30 years, it can be experienced by women at any age. It is diagnosed in 2-7% women who undergo surgery for acute pelvic pain[3].

The diagnosis of AT is important for preserving fertility. Early diagnosis and detorsion of the adnexa may preserve ovarian function and fertility. Although conventional methods are used for the diagnosis of AT, only 46% of patients who are diagnosed as having AT have real torsion during surgery and no specific diagnostic modality has yet been defined for the detection of AT[4]. The white blood cell (WBC) count increases during inflammatory processes in the body. It was shown that WBC count increases in cases of AT[5]. Many studies have shown that the neutrophil-to-lymphocyte ratio (NLR) is a significant inflammatory marker in various diseases[6,7]. The mean platelet (PLT) volume (MPV) is also important for the diagnosis of inflammatory states. The diagnostic importance of MPV has been shown in diseases such as acute appendicitis, pelvic inflammatory, and ectopic pregnancy[7-9]. Patients with pelvic pain due to adnexal causes should be diagnosed carefully. Ruptured or unruptured ovarian cysts (UOC) may cause pelvic pain. Clinical conditions requiring surgery should be differentiated from follow-up patients. The aim of this study was to investigate the diagnostic value of the NLR in the differential diagnosis of AT with ruptured or UOC.

Materials and Methods

Patients and data

A database of the 80 patients who underwent surgery for AT and ovarian cysts between January 2012 and June 2017 was retrospectively investigated. Patients with a suspicion of malignancy and tubal ovarian abscess were excluded from the study because malignancies and abscess formation may have an effect on blood count parameters. Three groups were formed [AT (n=35), UOC (n=25), and ovarian cyst rupture (OCR) (n=20)] and investigated. Demographic features, WBCs, neutrophil, lymphocyte, and PLT counts and hemoglobin (Hb) levels, NLR, MPV and red cell distribution width (RDW) before surgery were recorded. This study was approved by the Iocal Research Ethics Committee of Marmara University Faculty of Medicine the İnstitution (date: 09.2017 approval number: 543).

Statistical Analysis

Statistical analyses were performed using the SPSS 20.0 (SPSS, Version 20.0; Chicago, IL, USA) statistics software. In the study, descriptive and categorical data were evaluated as number (n) and percentage (%), and continuous data were studied as interquartile range and medians. The Mann-Whitney U test and Kruskal Wallis test were used for comparisons. The significance level was accepted as $p<0.05$.

Results

Demographics

The demographic findings of the groups were evaluated (Table 1). The median ages of the AT OCR, and UOC groups were 24, 32.5, and 34 years, respectively. There was a significant difference in respect to age and parity status of the patients. This difference was an expected feature of the AT cases, which are mostly seen at younger ages.

Complete Blood Counts

The median WBC count for the AT group was $12.1x10^3/\mu L$, OCR was $12.0x10^3/\mu L$, and the UOC was $7.3x10^3/\mu L$. There was a statistically significant difference between the groups ($p<0.001$). When the AT group was compared with the other groups individually, the AT group had no significant difference with the OCR group but had a significant difference with the UOC group ($p=0.0834$ and $p<0.001$). The median Hb levels of the AT, OCR, and UOC groups were 11.6 g/dL, 12.0 g/dL, and 12.5 g/dL ($p<0.001$). When further analysis was conducted, the statistically significant difference was due to the difference between the AT group and the UOC ($p<0.001$); no significant difference was noted between the AT and OCR groups. The median neutrophil counts for groups were found as $9.9x10^3/\mu L$ for the AT group, $9.9x10^3/\mu L$ for the OCR group, and $4.7x10^3/\mu L$ for the UOC group. The significant difference was attributable to the difference between the AT group and UOC group, rather than the difference between the AT and OCR groups ($p<0.001$ and $p=0.986$). The median lymphocyte count was $1.3x10^3/\mu L$ for the AT group, $1.4x10^3/\mu L$ for the OCR group, and $2.0x10^3/$

Table 1. Demographic findings

	AT		OCR		UOC		p
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
Age	24	19-31	32.5	28-37	34	29-46	<0.001
Gravida	0	0-1	1	0-2	1	0-4	0.08
Parity	0	0-1	1	0-2	1	0-2	0.03

AT: Adnexal torsion, OCR: Ovarian cyst rupture, UOC: Unruptured ovarian cysts

μL for the UOC group. When the AT group was compared with the OCR group, no statistically significant difference was found regarding lymphocyte counts (p=0.273), whereas there was a statistically significant difference between the AT and the UOC groups (p=0.009). The median RDWs, PLTs, and MPVs for all groups showed no statistically significant difference. The NLR was found to have a difference that reached statistical significance (p<0.001). When the groups were individually compared with the AT group, NLR was not found to be different to the OCR group (p=0.372), but there were a significant difference with the UOC group (p<0.001). Table 2 shows the complete blood count results and NLR of the three groups.

Discussion

AT has a frequency of 2.5 to 7.4% in emergency gynecologic operations. It is common in reproductive-age women and early diagnosis of this condition is vital for the preservation of the vitality of the ovary[10]. AT has nonspecific symptoms such as aches, nausea, vomiting, and low-grade fever, and as such, it is difficult to diagnose. OCR, ectopic pregnancy, adnexitis, acute appendicitis, gastroenteritis, and renal colic should be differentiated from AT[11]. The diagnosis of AT is generally based on ultrasonography. An increase in ovarian size, cyst of the adnexa, and free liquid in the pelvic cavity are the findings in ultrasound. However, ultrasound was shown to be normal in nearly half of all AT cases, especially in children[12]. Another diagnostic method is color doppler ultrasound, which is widely used for diagnosis of AT; doppler flow absence or decrease in ultrasound favors the diagnosis of AT. The detection of a normal blood flow pattern due to the dual blood supply of ovaries in 60% of cases of AT makes the role of doppler ultrasound in preoperative diagnosis debatable[13,14].

NLR has been a popular parameter for the diagnosis of several inflammatory and surgical conditions in which it has been shown to be superior to WBC counts[15]. NLR has also been shown to be useful in the diagnosis of many diseases concerning gynecologic inflammatory disorders and malignancies in several studies[16-18].

Pelvic pain requiring surgery due to adnexal pathologies may sometimes be a confusing issue for surgeons, especially in cases of OCR, which can be clinically followed up without a surgical intervention. Therefore, we aimed to investigate the role of NLR for the differential diagnosis of AT from OCR and UOC in this study.

Ercan et al.[19] compared preoperative NLR and WBC counts in patients with AT and ovarian cysts. They found an increase in both WBC and neutrophil counts in both groups, but a lower lymphocyte count in the ovarian cyst group. NLR was found to be significantly higher in the AT group compared with the ovarian cyst group[19]. We also found a significant difference in both WBC and neutrophil and lymphocyte counts and NLR of AT, OCR, and UOC. When the groups were individually compared, it was found that there was no significant difference between the OCR group and the AT group, whereas a significant difference was found between the AT group and the UOC group. In a similar study, AT and ovarian cyst groups were compared with several complete blood count markers. NLR, WBC, and neutrophil counts were found to be higher in the AT group compared with the ovarian cyst group. We also found the same difference, but as above, it was due to the UOC and AT group difference rather than the UOC and AT group difference. MPV and platelet counts were also investigated in the same study and no difference was found between the groups, similar to our findings[20]. OCR may need surgical intervention but can also be followed up clinically in hemodynamically stable cases. Observation with analgesia can usually be used for the management of hemorrhagic cysts and cyst rupture. Surgery should be performed in cases in which there is hemodynamic compromise, diagnostic uncertainty or suspicion of torsion, no relief of symptoms within 48 hours, and an increase in hemoperitoneum, and a decrease in HB level is detected[21].

Table 2. Complete blood count parameters and neutrophil-to-lymphocyte ratio

	AT		OCR		UOC		p
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
WBC (x10³/μL)	12.1	9.8-13.3	12.0	7.9-14.8	7.3	5.8-9.1	<0.001
HB (g/dL)	11.6	10.8-12.5	10.7	9.6-12.3	12.5	11.5-13.4	0.04
N (x10³/μL)	9.9	7.9-11.4	9.9	4.9-12.4	4.7	3.6-5.8	<0.001
RDW	13.4	12.8-14.4	14.6	13.4-15.5	13.4	13.0-14.1	0.126
PLT (x10³/μL)	262	206-334	218.5	190.5-286	258	211-284	0.157
MPV (fL)	8.9	7.7-9.3	9.0	8.3-9.4	8.5	7.9-8.9	0.243
L (x10³/μL)	1.3	0.8-1.9	1.4	1.0-1.8	2.0	1.6-2.4	0.016
NLR	8.0	4.0-14.1	7.5	3.7-11.5	2.2	1.8-2.7	<0.001

WBC: White blood cell, HB: Hemoglobin, N: Neutrophil, RDW: Red cell distribution width, PLT: Platelet, MPV: Mean platelet volume, L: Lymphocyte; NLR: Neutrophil-to-lymphocyte ratio, AT: Adnexal torsion, OCR: Ovarian cyst rupture, UOC: Unruptured ovarian cysts

Ruptured cysts and AT are different in respect to management, clinical follow-up of a ruptured cyst may be useful in specific cases, but early surgical intervention is important for preserving fertility in AT. NLR has been shown to be useful in differentiating ovarian cysts and AT[19,20]. Contrary to those findings, we found that NLR was not useful in the differential diagnosis of OCR and AT cases.

Study Limitations

The retrospective nature of the study is a limiting factor. Only data of patients of a single center were collected. Number of cases should be increased for to reach a clearer conclusion about the diagnostic value of neutrophil to lymphocyte ratio in differential diagnosis of adnexal torsion and ovarian cysts.

Conclusion

NLR can be used in the preoperative differential diagnosis of ovarian cysts and AT. However, the diagnostic value of NLR in the differentiation of OCR and AT cases was not determined in this study.

Ethics

Ethics Committee Approval: This study was approved by the Marmara University Local Ethics Committee (approval number: 543 date: 09.2017).
Informed Consent: Consent form was filled out by all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.S., R.B.B., Concept: S.S., Design: S.S., Data Collection or Processing: S.S., R.B.B., Analysis or Interpretation: S.S., R.B.B., Literature Search: S.S., Writing: S.S.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.86244

Turk J Obstet Gynecol 2018;15:95-8

Retrospective analysis of mature cystic teratomas in a single center and review of the literature

Matür kistik teratomların tek bir merkezde retrospektif olarak incelenmesi ve literatürün gözden geçirilmesi

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Abstract

Objective: The aim af this study is to evaluate patients with mature cystic teratomas (MCT) with regard to the view of updated knowledge using our retrospective findings.

Materials and Methods: This was a retrospective study and included a total of 306 patients from 2013 through 2017 at the İstanbul Zeynep Kamil Women and Children's Diseases Training and Research Hospital.

Results: The mean age of the patients was 34.03±11.98 years. Thirty (9.8%) patients were postmenopausal. Torsion was detected in 17 (5.6%) patients. There was a statistically significant relationship between MCT and CA 19-9 levels in our series ($p<0.01$) but no statistically significant correlation was found with other markers.

Conclusion: The possibility of malignancy at postmenopausal ages and in large MCT should not be forgotten. It should be kept in mind that MCT can be seen in unexpected places.

Keywords: Teratoma, CA19-9, adnexal masses

Öz

Giriş: Bu çalışmanın amacı matür kistik teratom (MKT) tanısı alan olguları, retrospektif bulgularımız ışığında güncellenmiş bilgiye bakış açısından değerlendirmektir.

Gereç ve Yöntemler: Bu, retrospektif bir çalışmadır ve 2013-2017 yılları arasında Zeynep Kamil Kadın ve Çocuk Hastalıkları Eğitim ve Araştırma Hastanesi'nde toplam 306 olgu içermektedir.

Bulgular: Olguların yaş ortalaması 34,03±11,98 idi. Otuz hasta (%9,8) postmenopozal idi. On yedi olguda (%5,6) torsiyon tespit edildi. Serimizde MKT ve CA 19-9 düzeyleri arasında istatistiksel olarak anlamlı bir ilişki vardı ancak diğer belirteçlerle istatistiksel olarak anlamlı korelasyon bulunmadı ($p<0,01$).

Sonuç: Postmenopozal yaş ve büyük MKT'lerde malignite olma ihtimali unutulmamalıdır. MKT'lerin beklenmedik yerlerde görülebileceği unutulmamalıdır.

Anahtar Kelimeler: Teratom, CA 19-9, adneksiyal kitle

PRECIS: Mature cystic teratomas has association with CA19-9.

Introduction

Mature cystic teratoma (MCT) is the most common ovarian tumor in women aged 20-30 years and accounts for 95% of ovarian teratomas[1]. Most MCTs are unilateral and benign. Only 10-17% of cases are bilateral[2]. MCTs include tissues of ectodermal (eg., skin, hair follicles, sebaceous glands), mesodermal (eg., muscle, urine) and endodermal origin (eg., lung, gastrointestinal)[3]. These tumors have a characteristic ultrasonic image and the specificity of ultrasound is 98-100%[4,5]. Malignant transformation is detected in 0.17-2% of MCTs. When it occurs, the most common is squamous cell carcinoma[6]. Risk factors for malignancy, age over 45 years, tumor size 10 cm and larger, rapid growth, and imaging findings (eg., low resistance tumor flow in Doppler)[2,7]. Early diagnosis and treatment of malignant transformation of MCT is very important because 5-year survival is only 15-30%[8]. We retrospectively analyzed preoperative, postoperative outcomes, and the clinicopathologic characteristics of mature cystic teratomas of the ovary at our institution.

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Received/Geliş Tarihi: 13.01.2018 **Accepted/Kabul Tarihi:** 18.03.2018

Materials and Methods

This was a retrospective study of all MCTs from 2013 through 2017. The data were obtained from the hospital database of İstanbul Zeynep Kamil Women and Children's Diseases Training and Research Hospital. It included a total of 306 cases. Data regarding age, size, laterality, gross morphologic features, levels of tumor markers alpha fetoprotein (AFP), cancer antigen (CA) 19-9, CA 15-3, carcinoembryonic antigen (CEA), and CA 125, complications, and surgery performed were retrieved from the hospital archives. A laparoscopic approach was preferred for patients who had no contraindications for laparoscopic surgery such as cardiac or pulmonary diseases, and no contraindications for being placed in the lithotomy position, but mainly the surgeon determined which surgery was to be performed.

Statistical Analysis

The IBM SPSS Statistics 22.0 program was used for the statistical analyses. When the study data were evaluated, the relationships between descriptive statistical methods (mean, standard deviation) was used. Besides these, Student's t-test was used for the comparison of two groups with normal distribution, and Spearman's correlation analysis was used to examine parameters without normal distribution. The chi-square test, Fisher's exact test, and Yates's continuity correction test were used for the comparison of qualitative data. Significance was assessed at $p<0.05$ level.

Results

The mean age of the patients was 34.03±11.98 years. Thirty patients (9.8%) were postmenopausal. The symptoms of the patients and the imaging methods used can be seen in Table 1. Torsion was detected in 17 (5.6%) patients. No cyst rupture was observed in any of the patients. Cyst diameters ranged from 1 to 20 cm with a mean of 6.68±6.0 cm. The mean CA 125 level was 22.96±15.5 U/mL, the mean CA 19-9 level was 71.11±17.8 U/mL, the mean CA 15-3 level was 13.97±13.5 U/mL, the mean CEA level 1.39±1.09 ng/mL, and the mean AFP level was 177.32±1.9 ng/mL. The normal values for CA 125 is 0 to 35 U/mL. The reference range of serum CA 15-3 is less than 30 U/mL. The reference range of serum CA 19-9 is less than 37 U/mL. The normal range for CEA in an adult non-smoker is <2.5 ng/mL, and for a smoker it is <5.0 ng/mL. An AFP level of less than 10 ng/mL is normal for adults. There was a statistically significant correlation between cyst diameter and CA 19-9 ($p<0.01$). As the diameter of the cyst increased, CA 19-9 level also increased. There was no statistically significant correlation between cyst diameter and other marker levels ($p>0.05$). When the distribution of localization was examined, 25 (8.2%) patients had bilateral localization and 281 (91.8%) had unilateral localization. Laparoscopic surgery was performed in 57.8% (n=177) of patients. In 19 (6.2%) patients, cyst rupture was detected during surgery, but none of the patients had chemical peritonitis in the post-operative period. Abundant lavage was applied to patients in whom rupture had occurred. Ninety-one cases (29.7%) were frozen and 9 cases (2.9%) were malignant. Three were reported as immature

Table 1. Symptoms and imaging methods of the patients

		n	%
Symptoms	Abdominal pain	117	38.2
	Abnormal uterine bleeding	61	19.9
	Incidental	123	40.2
	Postmenopausal bleeding	3	1
	Mass in labium majus	1	0.3
	Mass in vagina	1	0.3
Imaging methods	MRI	32	10.5
	USG	274	89.5

MRI: Magnetic resonance imaging, USG: Ultrasonography

Table 2. Localization of mature cystic teratomas and summary of the surgical procedure utilization

		n	%
Location	Bilateral	25	8.2
	Unilateral	281	91.8
	Right	133	43.5
	Left	146	47.7
	Labium majus	1	0.3
	Vagina	1	0.3
Surgery	Laparoscopy	177	57.8
	Laparotomy	127	41.5
	Mass excision from labium majus	1	0.3
	Mass excision from vagina	1	0.3
Type of surgery	Cystectomy	236	77.1
	Hysterectomy, bilateral salpingo oophorectomy	46	15
	Cystectomy, salpingo oophorectomy, omentectomy, bilateral pelvic and paraaortic lymphadenectomy	4	1.3
	Hysterectomy bilateral salpingo oophorectomy, omentectomy, bilateral pelvic and paraaortic lymphadenectomy	5	1.6
	Salpingo oophorectomy	13	4.2
	Hysterectomy unilateral salpingo oophorectomy	2	0.7

teratomas, three as squamous cell carcinoma, two as yolk sac tumor, and one as adenocarcinoma. The location of the MCTs and summary of the surgical procedures is presented in Table 2. The mean postoperative hospital stay was 1.89±1.06 days. Six (2%) patients had recurrence after 1 year. A dermoid cyst had been discovered during cesarean section in 8 patients.

Discussion

MCTs are usually diagnosed at reproductive ages and treatment is surgical. In this study, we observed an association with CA 19-9 and MCT, and we present 2 cases of MCT in unusual locations. Consistent with the literature, the mean age in our study was 34.03±11.98 years. In previous studies, it was found to present most commonly between the ages of 20-30 years[9]. In our study, we found that 91.8% of the MCTs were unilateral and more frequent on the left side (47.7%). The literature is unanimous with regards the unilaterality of the tumors, and there is no consensus on the right and left dominance. Some studies reported them more frequently on the right, some on the left[2,10-12]. Some 38.2% of the patients were admitted to hospital with symptoms of pain, and 40.2% cases were found incidentally. In parallel with the literature, MCTs could be detected in 16.6% and 75% of asymptomatic patients during routine physical examination and during any pelvic operations, respevtively[1,13-16]. The mean size of tumor was 6.68±6.0 cm. This is in accordance with previous studies where 60% of tumors were 5-10 cm in diameter[10,15-17]. Similar to previous studies, in our study, the most common complication of MCT was torsion and its detection rate was 5.6%. Malignant transformation of MCT was 2.9%. In the literature, malignant transformation is found between 1 and 3% and torsion rates are between 3.5 and 9.2%[6,13,14]. As supported by the literature, advanced age and tumor size are evaluated as risk factors for malignant transformation[2,7]. In our study, the mean diameter of MCTs with malignant transformation was 11.23 cm, and the mean age of patients with MCT with malignant transformation was 47 years. There was a statistically significant relationship between MCTs and CA 19-9 levels in our series ($p<0.01$) but no statistically significant correlation was found with other markers. In the literature, Ito[18] reported that MCTs has an association with CA 19-9 levels. However, Chen et al.[19], in contrast to our study, found that Ca 125, CA 153, and AFP together had an association with MCTs. With this study, we also evaluated 2 cases MCT that were rare in terms of their location. A 22-year-old patient presented with a vaginal mass of approximately 11 cm. It was filled with sebum and hair, and was diagnosed as MCT by the pathologists. Vural et al.[20] in 2015 concluded that the presence of vaginal teratoma was rare; only 8 cases have been reported worldwide. Our case was the largest of these cases. In another case, a 44-year-old woman was admitted to our hospital with a sebaceous cyst of about 3 cm in diameter in the labium majus, which was diagnosed as MCT in the pathology report. The rate of rupture during laparoscopy is very high in MCTs. Approximately 54% has been detected[21-23]. The probability of rupture has been found to be independent of size and location of MCT[24,25]. Cyst rupture was detected during surgery in 19 cases in our series in agreement with these studies. All cases of cyst rupture occurred during laparoscopy. However, we detected no chemical peritonitis postoperatively.

Study Limitations

The number of patients is small because it is a single-center experience.

Conclusion

The possibility of malignancy in women of postmenopausal age and in large MCTs should not be forgotten. It should be kept in mind that MCTs can be seen in unexpected places such as the eyelids, mouth, vagina, and labia majus. We observed an association with CA 19-9 and MCT, but further studies are needed.

Ethics

Ethics Committee Approval: This is a retrospective study.
Informed Consent: Consent form was filled out by all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.B.E., Ç.Y.A., Concept: Ç.Y.A., Design: Ç.Y.A., Data Collection or Processing: Ç.Y.A., Analysis or Interpretation: E.B.E., Literature Search: E.B.E., Writing: E.B.E.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study received no financial support.

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Clinical Investigation / Araştırma

DOI: 10.4274/tjod.12144

Turk J Obstet Gynecol 2018;15:99-104

Incidence of suspicious axillary lymph node involvement in fluorine-18 fluoro-D-glucose positron emission tomography/computed tomography in gynecologic cancers

Jinekolojik kanserlerde flor-18 floro-D-glukoz pozitron emisyon tomografisi-bilgisayarlı tomografide şüpheli aksiller lenf nodu tutulumunun insidansı

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Abstract

Objective: There is scarce information about axillary lymph node involvement in gynecologic cancers. We analyzed the incidence of suspicious axillary lymph nodes in gynecologic cancers.

Materials and Methods: We retrospectively analyzed the positron emission tomography/computed tomography findings of 251 patients with endometrial, cervical, and ovarian cancer. There is no cut-off value documented for axillary metastases from gynecologic cancers; therefore we adopted the cut-off standardized uptake values (SUVs) proclaimed in breast cancer.

Results: A total of 251 patients records were available for analysis; 40 patients (15.9%) with suspicious axillary lymph nodes were included in the study. Twenty-one and a half percent (n=20/93) of patients with endometrium cancer, 14.1% (n=14/99) of patients with ovarian cancer, and 10% (n=6/59) of those with cervical cancer had suspicious axillary lymph nodes. Patients with an maximum SUV (SUV_{max}) uptake higher than 3 underwent axillary lymph node biopsy. None of them was found to have axillary metastases of gynecologic cancers in the pathologic evaluation. In one patient with endometrial cancer, an obscure breast ductal carcinoma was diagnosed, another patient with endometrial cancer was found to have follicular lymphoma. The third patient with endometrial cancer had no malignancy in axillary lymph node biopsy, but had Hurthle cell neoplasia in a thyroid biopsy; the patient did not accept any surgical or medical treatment for endometrial cancer and died 23 months later. There were three (7.5%) metachronous cancers out of 40 gynecologic cancers; two patients were explained above, the third patient with endometrium cancer, who was not histopathologically evaluated although the axillary SUV_{max} was <3, had rectosigmoid cancer and glioblastoma metachronously.

Conclusion: Our study shows that an important ratio (14-21%) of patients with gynecologic cancer has suspicious axillary lymph nodes. Increased SUV_{max}, particularly above 3, might be used as an indication for axillary biopsy and may help to identify secondary metastatic cancer.

Keywords: Gynecologic cancers, maximum standardized uptake value, positron emission tomography/computed tomography, metachronous, axillary lymph node

Öz

Amaç: Jinekolojik kanserlerde aksiller lenf nodu tutulumunun prognostik önemi ve tedavisi açısından kısıtlı bilgiler mevcuttur. Bu çalışmanın amacı, jinekolojik kanserlerde şüpheli aksiller lenf nodu insidansını değerlendirmektir.

Gereç ve Yöntem: Kliniğimize başvuran toplam 251 endometrium, serviks veya over kanseri tanısı konulmuş hastaların pozitron emisyon tomografisi-bilgisayarlı tomografi bulgularını retrospektif olarak analiz ettik. Jinekolojik kanserler için aksiller metastazı açıdan belgelenen herhangi bir sınır değer belirlenmemiş olması nedeniyle çalışmamızda meme kanseri için belirlenen standart uptake değerini (SUV) sınır değer olarak kabul ettik.

Bulgular: Analizler için toplam 251 hasta kaydı mevcuttu. Şüpheli aksiller lenf nodu olan 40 hasta (%15,9) çalışmaya dahil edildi. Endometrium kanseri olan hastaların %21,5'inde (n=20/93), over kanseri olan hastaların %14,1'inde (n=14/99) ve serviks kanseri olan hastaların %10'unda

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Received/Geliş Tarihi: 28.11.2017 **Accepted/Kabul Tarihi:** 26.04.2018

(n=6/59) şüpheli aksiller lenf nodu tespit edildi. Maksimum SUV (SUV_{maks}) değeri 3'ten yüksek bulunan 5 hastada aksiller lenf nodundan biyopsi alındı. Patolojik değerlendirmede hiçbir hastada primer jinekolojik kansere ait aksiller metastaz saptanmadı. Endometrium kanseri olan bir hastada invaziv meme karsinomu, endometrium kanseri olan başka bir hastada foliküler lenfoma tanısı konuldu. Endometrium kanseri olan üçüncü hastada aksiller lenf nodu biyopsisinde tümör tespit edilmedi ancak tiroid biyopsisinde Hurthle hücre neoplazisi saptandı, bu hasta endometrium kanseri için herhangi bir cerrahi veya medikal tedaviyi kabul etmedi ve 23 ay sonra hayatını kaybetmiştir. Çalışmamıza şüpheli aksiller lenf nodu ile dahil olan 40 hastanın 3'ünde (%7,5) metakron kanser izlendi; iki hasta yukarıdaki paragrafta açıklandı, üçüncü ve endometrium kanser tanısı olan hastada rektosigmoid kanseri ve glioblastoma metakron olarak izlendi, bu hastaya SUV_{maks} <3 olması nedeniyle aksiller lenf nodu biyopsisi yapılmamıştır. **Sonuç:** Çalışmamızda, jinekolojik kanserli hastaların önemli bir oranının (%14-21) şüpheli lenf nodu gözlenmektedir. SUV_{maks} özellikle 3'ün üzerinde olan olgularda aksiller lenf nodu biyopsisi yapılması muhtemel ikincil metastatik kanserin belirlemesinde yardımcı olabilir.
Anahtar Kelimeler: Jinekolojik kanserler, maksimum standart uptake değeri, pozitron emisyon tomografisi/bilgisayarlı tomografi metakron, aksiller lenf nodu

PRECIS: In gynecologic cancers, patients with suspicious axillary lymph nodes need to be evaluated and further investigated to exclude other causes.

Introduction

Two-deoxy-2-[fluorine-18] fluoro-D-glucose (^{18}F-FDG) positron emission tomography (PET) adds valuable data based on the increased glucose uptake and depicts metabolic abnormalities before morphologic alterations occur. PET has been widely used in staging, pre-operative planning, and follow-up of gynecologic cancers in our department, as well as worldwide. Whole-body acquisition by ^{18}F-FDG PET/computed tomography (CT) imaging may demonstrate unusual findings in distant unexpected localizations[1]. Patients with a gynecologic malignancy are at greater risk of developing synchronous or metachronous secondary cancers, therefore these unusual ^{18}F-FDG PET/CT findings may be important. Besides, skip metastases to the axillary lymph nodes may influence surgical and adjuvant treatment of the patient. We have noticed that in an important part of patients with gynecologic cancer, ^{18}F-FDG PET/CT has indicated incidental axillary lymph nodes although axillary lymphadenopathy is rare in gynecologic cancers[1]. There is a paucity of information about axillary lymph node involvement in gynecologic cancers in terms of prognostic importance and accurate management. The aim of the present study was to analyze the incidence of suspicious axillary lymph nodes in gynecologic cancers and evaluate the oncologic and ^{18}F-FDG PET/CT features.

Materials and Methods

We retrospectively analyzed the ^{18}F-FDG PET/CT findings of 251 patients with endometrial, cervical, and ovarian cancer who were referred to our clinic between 2010 and 2017. We included gynecologic oncology patients with suspicious axillary lymph nodes when they had one of the following features in ^{18}F-FDG PET/CT imaging: lymph nodes with a diameter equal to or larger than 10 mm, parenchymal thickening and loss of fatty hilum, or increased FDG-uptake value equal to or greater than 1.8. There is no cut-off value documented for axillary metastases from gynecologic tumors; therefore, we adopted the cut-off standardized uptake value (SUV) proclaimed in breast cancer. However, there is also no consensus for SUV values for the detection of axillary metastases in breast cancer. An SUV cut-off value of axillary metastases in breast cancer set at 1.8 or more is reported to have 35.6% sensitivity, 100% specificity, and 100% positive predictive value[2]. In other studies, the optimal cut-off level of maximum SUV (SUV_{max}) on PET/CT for malignant isolated axillary lymph nodes was reported as 3.01[3]. Women with an SUV uptake higher than 3 underwent histopathologic evaluation through fine-needle core biopsy, excisional biopsy or complete axillary lymph node dissection according to the surgeon's preference. Demographic and oncologic characteristics of the patients, ^{18}F-FDG PET/CT findings, SUV_{max}, follow-up of axillary lesions, and biopsy results were recorded. FDG-PET image acquisition and whole-body FDG-PET scans were performed as described using the Philips Gemini TF PET/CT scanner (Philips Medical Systems B.V., Eindhoven, The Netherlands). Patients were prepared with a 6 h fast because serum glucose levels had to be <150 mg/dL prior to glucose tracer administration. At 60 min after the intravenous injection of 3.7 MBq/kg (0.1 mCi/kg) ^{18}F-FDG (Monrol, Eczacıbaşı, İstanbul, Turkey), PET/CT was performed. Subsequently, an emission scan was recorded in the three-dimensional mode following CT for 2 min per position. PET and CT images were examined in the cross-sectional planes view and in the rotating maximum-intensity projection. The study was approved by the Süleyman Demirel University Local Ethics Committee (approval number: 175 dated 04.10.2017).

Statistical Analysis

Statistical analyses were performed using the MedCalc Software (version 17.4.4, Belgium) and IBM SPSS Statistics 24 Software. One-way ANOVA and the chi-square test were used to compare variables. A p value of 0.05 or less was defined as statistical significance.

Results

A total of 251 patient records were available for analyses. Forty (15.9%) patients with suspicious axillary lymph node metastases were included in the study. The mean age of patients was 60.4±9.1 years. Axillary lymph node involvement

was most commonly observed in patients with endometrium cancer. There were 93 patients with endometrium cancer and a suspicious lymph node was found in 21.5% (n=20). There were 99 patients with ovarian cancer and 14.1% (n=14) had a suspicious axillary lymph node in ^{18}F-FDG PET/CT imaging. Ten percent (n=6/59) of patients with cervical cancer had suspicious axillary lymph nodes (Table 1). In 17 patients (out of 40), suspicious axillary findings were evident in pre-operative/treatment PET/CT. Twenty-three patients (out of 40) had no suspicious axillary lymph nodes in preoperative/treatment PET/CT; suspicious axillary lymph nodes were found as a new findings in the follow-up of these patients (Table 2). In follow-up of the 40 patients with suspicious axillary lymph nodes, 28 (70%) patients had regression, 3 (7.5%) progressed, and 9 (22.5%) patients remained stable. Of the three progressive cases, one patient with endometrial cancer did not accept any surgical or medical treatment for endometrial cancer and died 23 months after ^{18}F-FDG PET/CT imaging. The second patient had stage IIIB ovarian cancer and received 6 courses of carboplatin-paclitaxel chemotherapy (3 neoadjuvant and 3 adjuvant); the suspicious lymph node regressed after chemotherapy. The third patient with progressive suspicious axillary lymph node had stage IIIC ovarian cancer, she received 3 courses of carboplatin + paclitaxel chemotherapy before surgery, and was followed up with 36 courses of chemotherapy after surgery, (6 courses of carboplatin + paclitaxel, 6 courses of bevacizumab, 6 courses of carboplatin + paclitaxel, 6 courses of gemcitabine, 6 courses

Table 1. Characteristic of gynecologic oncology patients with suspicious axillary imaging in positron emission tomography/computed tomography

	Endometrium cancer	Ovarian cancer	Cervical cancer	p
	n=93	n=99	n=59	
Age	61.3±7.8	57.2±10.6	64.6±8.8	>0.05
Suspicious axillary lymph node (n)	20 (21.5%)	14 (14.1%)	6 (10.1%)	>0.05
FDG uptake	9/20	7/14	3/6	>0.05
Parenchymal thickening and loss of fatty hilum in lymph nodes with a diameter ≥10 mm, no FDG uptake	11/20	7/14	3/6	>0.05

FDG: Fluoro-D-glucose

Table 2. Positron emission tomography/computed tomography features, maximum standardized uptake value values, and results of a suspicious axillary lymph node imaging

Diagnosis of suspicious axillary lymph node in	
Pre-treatment first PET/CT	17/40 (42.5%)
Follow up PET/CT as a new finding	23/40 (57.5%)
Mean SUV_{max}	2.4±2.3
SUV_{max} cut-off	
No FDG uptake	21 (52.5%)
<1.8	2 (5%)
>1.8	17 (42.5%)
Follow-up of suspicious axillary lymph nodes	
Regression	28 (70%)
Progression	3 (7.5%)
Stable disease	9 (22.5%)

SUV_{max}: Maximum standardized uptake value, PET/CT: Positron emission tomography/computed tomography, FDG: Fluoro-D-glucose

Table 3. The treatment and follow-up of 40 patients with suspicious axillary lymph node in positron emission tomography/computed tomography

	Stage	n	Treatment
Endometrial cancer	IA	7	Follow-up
	IB	3	Radiotherapy: 2 patients Follow-up: 1 patient
	II	5	Radiotherapy: 4 patients Radiotherapy and chemotherapy: 1 patient
	IIIC1	1	Chemotherapy
	IIIC2	1	Radiotherapy and chemotherapy
	IVB	2	Chemotherapy: 1 patient Refused surgical or medical treatment for endometrial cancer: 1 patient
	?	1	After diagnosis with endometrial biopsy she has had no follow-up and treatment in our center
Ovarian cancer	IIC	2	Chemotherapy
	IIIA	1	Chemotherapy
	IIIB	4	Chemotherapy: 1 patient Radiotherapy and chemotherapy: 3 patients
	IIIC	5	Chemotherapy
	IV	1	Chemotherapy
	Serous borderline	1	Follow-up
Cervical cancer	IA2	1	Follow-up
	IB1	1	Radiotherapy and chemotherapy
	IVA	2	Radiotherapy and chemotherapy
	IVB	2	Radiotherapy and chemotherapy

?: After diagnosis with endometrial biopsy she hasn't had any follow up and treatment in our center

Table 4. Deceased patients according to diagnostic subtype

Disease	n			Average survival (months)	p value	Description
Endometrial cancer	6	SUV_{max}	No FDG uptake (n=4)	43.7 (±21.0)		Three died of their cancer and 1 of other reasons
			<1.8 (n=0)	0		
			>1.8 (n=2)	20 (±4.2)	>0.05	One died of her cancer and 1 of other reasons
Ovarian cancer	10	SUV_{max}	No FDG uptake (n=5)	43.8 (±23.4)		Died of cancer
			<1.8 (n=0)	0		
			>1.8 (n=5)	42.8 (±11.9)	>0.05	Died of cancer
Cervical cancer	2	SUV_{max}	No FDG uptake (n=2)	35.5 (±26.1)		Died of cancer
			<1.8 (n=0)	0	-	
			>1.8 (n=0)	0		

SUV_{max}: Maximum standardized uptake value, FDG: Fluoro-D-glucose

Table 5. Patients who had axillary lymph node standardized uptake value uptake >3 had intervention for histopathologic evaluation. Features of patients who had an axillary needle core biopsy. No patient was found to have axillary metastases in the pathologic evaluation

Patient	Primary disease	Age	Management of primary tumor	SUV_{max} of axillary lesion	Other positive findings in PET/CT	Biopsy method	Biopsy result	Alive/dead
1	Endometrium cancer, mix endometrioid-mucinous histology, grade 1 stage IA	61	Hysterectomy +salpingo-oophorectomy +pelvic and para-aortic lymph node dissection	10.46	Supraclavicular lymphadenopathy, splenic hilar lymphadenopathy, splenic hypermetabolic lesion, gastric lesion	Excisional biopsy and axillary dissection	Follicular lymphoma	Alive
2	Endometrium cancer, endometrioid histology, grade 1 stage IA	75	Hysterectomy +salpingo-oophorectomy +pelvic lymph node dissection	6.16	Suspicious mass in right breast with SUV uptake 4.09	Fine needle core biopsy followed by right quadrenectomy and axillary dissection	Breast ductal carcinoma metastasis	Alive
3	Endometrium cancer, mix endometrioid-mucinous histology, grade 1, stage IA	67	Hysterectomy +salpingo-oophorectomy +pelvic and para-aortic lymph node dissection	5.49	6 mm nodulary lesion in right lung hypermetabolic mediastinal lymph nodes	Fine needle core biopsy	No tumor	Alive
4	Endometrium cancer	74	Refused surgical or medical treatment for endometrial cancer	4.72	Hypermetabolic nodulary lesion in thyroid gland (SUV_{max} 7.7)	Fine needle core biopsy of axilla and thyroid	No tumor in axilla and Hurthle cell neoplasia in thyroid	Died 23 months after PET/CT imaging
5	Cervical cancer, squamous cell, stage IB2	68	Hysterectomy +salpingo-oophorectomy	4.29	Interlobar hypermetabolic lymphnodes in both lungs	Axillary dissection	No tumor	Alive

SUV_{max}: Maximum standardized uptake value, PET/CT: Positron emission tomography/computed tomography

of topotecan, 6 courses of doxorubicin); however, despite this intense chemotherapy, the disease progressed and she died after 42 months' survival. The treatment and follow-up of 40 patients with the suspicious axillary lymph node in PET-CT is shown in Tables 3 to 5. In our last follow-up, 18 patients out of the 40 had died, 16 of which of their cancer, and 2 of other causes (Table 4). Eighteen of the remaining 22 patients were in remission, 3 patients had progression, and 1 patient was lost to follow-up. Twenty-one of the 40 patients had no FDG uptake but had obviously enlarged axillary lymphadenopathy in ^{18}F-FDG PET/CT imaging, and 19 patients had FDG uptake in the axilla (Table 1, Figure 1, 2). The mean SUV_{max} in axillary lymph nodes with FDG uptake (SUV_{max}) was 2.4±2.3. Forty-two percent of patients had an SUV_{max} higher than the cut-off value of 1.8 (Table 2). Patients with an SUV_{max} uptake higher than 3 underwent axillary lymph node biopsy. There were 6 patients with an SUV_{max} >3. All but one gave informed consent for an intervention for histopathologic verification. None of the patients was found to have axillary metastases in the pathologic evaluation. In one patient with endometrial cancer, an obscure breast ductal carcinoma was diagnosed, the patient had stage IIIC breast cancer with axillary metastasis. She underwent surgical treatment for breast cancer along with surgical treatment for endometrial cancer. She received adjuvant chemo-radiotherapy for breast cancer and was disease-free in her last follow-up. The second patient was found to have follicular lymphoma and sent to hematology after surgical treatment of endometrial cancer. The third patient with endometrial cancer had no malignancy in her axillary lymph node biopsy, but had Hurthle cell neoplasia in a thyroid biopsy; she refused surgical or medical treatment for endometrial cancer and died 23 months later. The other patients who underwent axillary lymph node biopsy had no malignancy at the pathologic evaluation. In the sixth patient with cervical cancer; initially, there was a suspicious lymph node with an SUV_{max} equal 3.64, which was interpreted as reactive, but it could not be observed in the second ^{18}F-FDG PET/CT imaging after the first-line chemo-radiotherapy before the biopsy. In the last follow-up, she was in remission. The features of patients who underwent axillary biopsy are shown in Table 5.

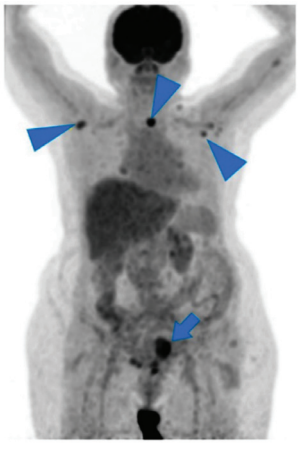

Figure 1. Positron emission tomography/computed tomography maximum intensity projection image shows increased fluoro-D-glucose uptake in uterine cavity (arrow), bilateral axillary lymph nodes and nodular lesion in left thyroid lobe (arrowhead)

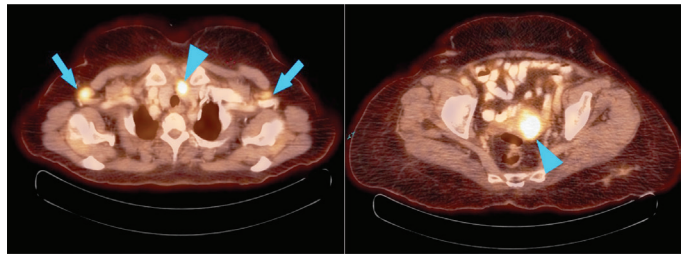

Figure 2. a) Positron emission tomography/computed tomography fusion image of the thorax. The image shows bilateral axillary hypermetabolic axillary lymph nodes [maximum standardized uptake value (SUVmax): 4.72] (arrow), a hypermetobolic nodular lesion in the left lobe of the thyroid was found incidentally (SUVmax: 7.72) (arrowhead). Fine-needle aspiration of thyroid revealed Hurthle cell neoplasia, b) Hypermetabolic mass in the uterus with a SUVmax value of 9.42 (arrowhead)

Discussion

We found that an important proportion of gynecologic oncology patients had suspicious lymphadenopathy in ^{18}F-FDG PET/CT imaging, in particular, a significant proportion of patients with endometrium and ovarian cancer (21.5% and 14.1%, respectively). In our study, 52.5% of these patients had enlarged axillary lymph nodes without FDG uptake and 47.5% of these patients had enlarged axillary lymph nodes with high SUV uptake. The mean axillary SUV_{max} was 2.4±2.3. PET–CT is reported to have low sensitivity but high specificity in breast cancer, which indicates that it is more useful when metastasis is suspected. There is currently no consensus regarding the differentiation of benign from malignant lymph nodes and there is also no agreement on the cut-off for SUV_{max} values[4]. Axillary lymph node metastases are usually expected in primary tumors of the breast, lung, thyroid, stomach, skin, and ovary[5]. The most common source of axillary lymph node metastasis is breast cancer, metastasis from non-mammary primary cancer to the axillary lymph nodes is less than 3%[6]. Metastases from a gynecologic malignancy are considered extraordinary. Axillary lymph node metastases in endometrium cancer are reported as 0.03% by Aalders et al.[7]. Axillary involvement in ovarian cancer has been reported as case series and is usually associated with breast metastases[8]. Serous histology in ovarian cancer was the most important risk factor for axillary metastasis. Euscher et al.[9] studied 35 patients with ovarian, fallopian tube or peritoneal serous carcinoma that presented as lymphadenopathy. In their study, there were only 2 axillary lymphadenopathies with peritoneum primary sites[9]. Sangle et al.[10] reported a 63-year-old patient who had serous carcinoma of the fallopian tube with axillary lymph node involvement. Isolated axillary lymph node metastasis from serous ovarian cancer has also been published as case reports in the literature[11,12]. Skagias et al.[8] reported a patient with ovarian carcinoma that presented with axillary

lymph node metastasis. Sanuki et al.[5] reported a 57-year-old woman with endometrium cancer with axillary lymph node involvement in Japan in 2007. As a determination of their study to evaluate a cut-off value regarding the differentiation of benign from malignant lymph nodes, Kyoung[3] reported that axillary lymph nodes with isolated FDG uptakes in whole-body PET/CT showed a low risk of malignancy (25%), and axillary lymph nodes with an SUV greater than 3.01 were malignant. Therefore, in our protocol, we suggested histopathologic evaluation of axillary lymph nodes in patients with an SUV_{max} >3. In our study, 6 patients had an SUV_{max} higher than 3. All but one gave informed consent for an intervention for histopathological verification. However, management and follow-up for axillary lymph nodes with abnormal visual and functional findings in PET/CT in patients with endometrial or ovarian cancer are not consistently established. It is uncommon to detect a synchronous or metachronous secondary cancer in reproductive system malignancies. The incidence of occult breast cancer is reported as 0.3-1% of all patients with breast cancer. Occult breast cancer is the most likely diagnosis associated with axillary metastasis[13]. Sughayer et al.[14] reported a 63-year-old woman with collision axillary metastasis from breast cancer and ovarian cancer. Atallah et al.[6] reported a case of ipsilateral breast cancer and occult tubal serous carcinoma. In our study, there were 3 (7.5%) metachronous cancers out of 40 gynecologic cancers with suspicious axillary lymph nodes in PET/CT imaging, which is reported as uncommon in previous literature. The first patient with endometrial cancer, an obscure breast ductal carcinoma, was diagnosed through axillary lymph node biopsy, she had stage IIIC breast cancer with axillary metastasis. The second patient was found to have follicular lymphoma via axillary lymph node biopsy and was sent to hematology after surgical treatment for endometrial cancer. The third patient, who had stage IB endometrial cancer, was not histopathologically evaluated because of having an axillary SUV_{max} <3, had rectosigmoid cancer and glioblastoma metachronously. This patient died of glioblastoma. This high metachronous cancer ratio warrants detailed evaluation of patients with gynecologic cancer with axillary lymph node abnormalities in ^{18}F-FDG PET/CT for secondary primary cancer.

Conclusion

Our study shows that an important ratio (14-21%) of patients with gynecologic cancer has suspicious axillary lymph nodes. Increased SUV_{max}, particularly higher than 3, might be used as an indication for axillary lymph node biopsy and may help to identify secondary metastatic cancer. Axillary metastases in gynecologic cancers may up-stage the patient to stage IV and may preclude aggressive cytoreductive surgery or may ensure adjuvant chemotherapy. Patients with increased SUV uptake higher than 3 in ^{18}F-FDG PET/CT imaging need to be evaluated, and further investigations should be performed to exclude other causes of lymphadenopathy.

Ethics

Ethics Committee Approval: The study was approved by the Süleyman Demirel University Local Ethics Committee (approval number: 175 dated 04.10.2017).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.E., Concept: J.R., S.C.İ., E.E., Design: J.R., S.C.İ., E.E., E.E., Data Collection or Processing: J.R., E.E.Ö., C.H., Analysis or Interpretation: J.R., E.E.Ö., C.H., S.S.Ç., E.E., Literature Search: J.R., E.E.Ö., C.H., E.E., E.E., Writing: J.R., E.E.Ö., C.H., E.E.

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

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

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Review / Derleme

DOI: 10.4274/tjod.33407

Turk J Obstet Gynecol 2018;15:105-11

A mini-review of aesthetic gynecology and leading gynecology associations' approaches to this issue

Estetik jinekoloji ve önde gelen jinekoloji derneklerinin konuya yaklaşımı hakkında mini derleme

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Abstract

Aesthetic gynecology has seen increasing patient and physician demand. Although this typically falls in the reign of obstetrics and gynecology, plastic surgeons and cosmetic surgeons have also developed great interest in this field. Currently, few if any obstetrics and gynecology residency or fellowship programs teach this subject matter though inroads have taken place in plastic surgery and cosmetic surgery training programs that had the foresight to include specific training in this field. Currently, many surgeons start by first training in various established certification and preceptorship programs based in the United States and the United Kingdom. New programs worldwide in 2016-2017 have also been launched to offer certification training to interested physicians in both surgical and non-surgical treatments. A steady flow of certificate programs continues to evolve in Turkey, the Middle East, Spain, and South America, as a second wave of experts emerge. We present a review of surgical and non-surgical techniques of what is presently called "aesthetic gynecology" and the approaches of prominent gynecologic societies regarding this relatively new subspecialty.
Keywords: Aesthetic gynecology, labiaplasty, vaginoplasty, rejuvenation, radiofrequency

Öz

Estetik jinekolojiye olan talep hem hastalar hem hekimler tarafından gün geçtikçe artmaktadır. Bu alan kadın hastalıkları ve doğum uzmanlarının konusu olmasına rağmen, plastik cerrahlar ve kozmetik cerrahlar da bu alana büyük ilgi göstermektedirler. Günümüzde, kadın hastalıkları ve doğum asistanlığında veya burs programlarında eğitim verilse de bu alanda spesifik eğitim vermek için oluşturulmuş plastik cerrahi ve kozmetik cerrahi eğitim programlarında da ilerleme kaydedilmiştir. Halen pek çok cerrah, ilk eğitimlerini Birleşik Devletler ve Birleşik Krallık'taki çeşitli sertifikasyon ve eğitmenlik programlarından almaktadırlar. 2016-2017 yılları arasında dünya çapında yeni programlar, cerrahi ve cerrahi olmayan prosedürlerle ilgilenen hekimlere sertifikasyon eğitimi sunmuştur. Türkiye, Orta Doğu, İspanya ve Güney Amerika'da da ikinci uzman dalgası oluşturacak sertifika programları açılmaya devam etmektedir. Bu derlemede günümüzde "estetik jinekoloji" olarak adlandırılan cerrahi ve cerrahi olmayan teknikleri ve bu nispeten yeni sayılan alt uzmanlık alanıyla ilgili önde gelen jinekoloji derneklerinin yaklaşımlarını gözden geçireceğiz.
Anahtar Kelimeler: Estetik jinekoloji, labioplasti, vajinoplasti, rejuvenasyon, radyofrekans

Introduction

Developments in both technology and fashion induce seasonal changes in the notion of beauty. The social and cultural differences among countries also play a highly significant role in this matter. Thus, one cannot give an exact description of the normal view of external genitalia. However, upon consideration of anatomic variations, Hodgkinson and Hait[1] defined the ideal aesthetic picture of female external genitalia as the one in which the labia minora are small and not larger than the labia majora. The Motakef classification is based on the protrusion of the labia minora that exceeds the size of the labia majora[2]. The Banwell classification categorizes labia according to their shape and morphologic variations[3]. None of the classification systems have been accepted by gynecologic or plastic surgical societies and are rarely used. Apart from medical indications such as labial hypertrophy and congenital adrenal hyperplasia, most operations are performed upon the patient's request due to a feeling of enlargement and looseness in the vagina, a desire to improve sexual function, discomfort when wearing clothes or doing fitness activities, or with an aim to increase sexual satisfaction for both herself and her partner. Regarding

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Received/Geliş Tarihi: 24.01.2018 **Accepted/Kabul Tarihi:** 02.03.2018

anxiety about external appearance, motives for surgery include perceived larger size or asymmetry of the labia minora and a dark-colored appearance of the labia majora[4,5]. Although it is not possible to define the ideal aesthetic genitalia, patient-specific techniques chosen based on the patient's anatomy and applied with a realistic approach can increase patient satisfaction and reduce complication rates.

Aesthetic gynecology surgical techniques

Vaginal tightening, or vaginoplasty, refers to surgery of the vaginal entrance, deeper canal, and epithelium. This procedure is not the same as pelvic floor repair and if there is pelvic floor defect it should be part of urogynecologic assessment. Frequently, it is considered as a modification of standard posterior repair but may include excision of lateral vaginal mucosa or high posterior repair[6,7]. It is usually carried out along with perineoplasty and paravaginal repair,with or without an anterior colporrhaphy,to adress patient concerns of having a large or loose vagina. From the perspective of cosmetic gynecology, the surgeon must determine the limits of the planned vaginal diameter reduction, in advance, in dialogue with the patient, and keep in mind that the patient's expectation might be unrealistic. The risks of over-tightening must be explained. Perineoplasty is a specific repair of the vaginal entrance and the space between the vaginal and the anal openings. It is a complementary procedure to prolapse surgery. The surgical goals are cosmetic achievement through reformation of the perineal body, thereby lifting the perineum, and greater sexual satisfaction through increased friction with penile penetration. Also it can straighten out the path that stool passes through and improve defecation mechanics. However, when perineoplasty is applied alone to ameliorate sexual dysfunction, conclusive results indicating successful application are lacking[4]. Labiaplasty is the most commonly applied aesthetic genital surgery and should be aimed at improving the appearance of the external genitalia and reducing obstructive tissues during intercourse. The surgical reduction of the labia minora was first described as an aesthetic motive in the plastic surgery literature by Hodgkinson and Hait[1]. When we look at the literature, many techniques have been described for labiaplasty: deepithelialized reduction, linear incision, composite reduction, wedge reduction, W-plasty excision or Z-plasty[2]. The linear excision technique is the most preferred among gynecologists because of its simple and minimally invasive approach. In Laguna Beach, California, terms that are used for the labial reductions according to the level of labia majora below or at the same level with labium minora: rim look, Barbie look or hybrid look[8]. With this technique, smoother contours can be achieved. According to Miklos and Moore's[5] study, 97% of 550 women wanted to remove dark edges. If the patient wants to retain the dark edges of the labium minora, a wedge technique can be performed. This technique is mostly performed by plastic surgeons. There are modifications of this technique; central V-plasty, and 90-degree Z-plasty[9-11]. If any of these wedge techniques are performed, perfect hemostasis is very important to prevent wound dehiscence and fistula formation. Although the wedge resection technique preserves a more natural edge look, most women want removal of this irregular labial edge for a smoother and more petite appearance (Figure 1a, b). If a minimal amount of labium needs to be excised deepithelialized reduction technique may be preferred[12]. If the deepithelialized area is large, it can result in increased labial thickness and a visible suture line[13]. Labia majora reduction, or labia majoraplasty, is intended to reduce the size of labia majora that appear saggy and hyperplastic. Here, the excess skin, and, if necessary, fat pad is removed. In over 90% of cases, only segments of the labia majora skin is removed. Longitudinal resection with scar placement between the thigh-vulva crease or resection with scar placement between the labium majora and minora can be performed. The first technique has high risk for wound dehiscence because of scar formation in a high tension area[14].

The labia majoraplasty procedure is employed to address medical conditions such as congenital lymphedema and sagginess from chronic steroid use in conditions such as congenital adrenal hyperplasia. When performed for aesthetic concerns, labia minoraplasty can be performed in conjunction with labia majoraplasty, and are typically separate procedures (Figure 2a, b). Clitoral hood reduction (hoodoplasty) is performed with labia minoraplasty, but not always. One of the techniques is to reduce the clitoral hood skin over the clitoris using the skinning deepithelialization technique, lateral in location. A

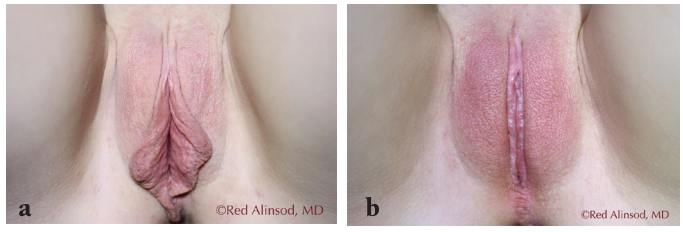

Figure 1. Young lady in her 20s felt uncomfortable with her labia and redundant and loose perineal tissues. She requested an aggressive labiaplasty for both comfort and personal confidence a) before surgery, b) two months after surgery (labia minoraplasty, clitoral hood reduction, and perineoplasty)

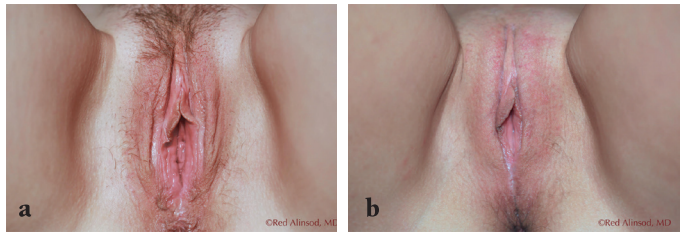

Figure 2. Middle-aged multiparous female, unhappy with the appearance of her loose labia majora and her introitus. She requested a labia majoraplasty and perineoplasty and declined vulvar filling procedures a) before labia majoraplasty: front view, b) months after labia majoraplasty: front view

vertical clitoral hood reduction technique is performed for wide hoods and redundant hoods with multiple skin folds. The composite reduction technique is a type of minoraplasty and it also achieves clitoral protrusion correction[15]. The clitoris is not unhooded in the large majority of cases and the procedure is performed to achieve better symmetry and reduce the "top-heavy" look post labia minoraplasty. Goodman[7] defined the goal of this reduction as improving sexual arousal by revealing more of the clitoris.

Labia Majora augmentation is not considered to be a surgical technique, its purpose is to aesthetically improve labia majora with a hypoplastic or loose appearance. To this end, autologous fat-grafting or hyaluronic acid (HA) fillers are mostly used. Although the region selected for autologous fat grafting may be any area with abundant fatty tissue, the most frequently used regions are the thigh and the inner part of the knee. The fat graft is prepared through techniques such as a washing centrifuge; the Coleman[16] technique is the most frequently used. For an autologous fat graft, the injection must take into account re-absorption, otherwise a second session may be required to achieve the desired result. The use of HA as a filler is also a frequently employed technique in non-surgical rejuvenation. Fat is the predominant product used in the United States of America (USA) and HA is predominant in Europe due to costs. In the USA, filling of the labia majora is not as popular as in Europe. The preference for American women is a sleeker and more petite appearance, which labia majoraplasty or radiofrequency (RF) shrinkage can help achieve. In Europe, there is more use of filling techniques, specifically HA. The outcome is determined by the surgeon's anatomic knowledge and operating skills. However, the use of HA in this region must be carefully considered. Proper techniques and materials can prevent inflammatory developments, such as a granuloma formation.

Hymenoplasty involves many ethical issues. It is known as revirgination in Western countries and is a socio-cultural issue, especially in Muslim countries. The operation itself is the least-studied female genital procedure. It is most accurate to classify hymenoplasty as a reconstructive procedure and exclude it from the set of aesthetic procedures. The ethics committees of many associations do not consider it as cosmetic genital surgery[17,18]. This surgery can be life-saving for women. It is a simple procedure and can be described as a "vaginal repair" for the patient's protection of privacy.

Aesthetic gynecology non-surgical techniques

Laser treatment for vaginal laxity: several fractional lasers have been used for non-invasive treatment of vaginal laxity. Fractional carbon dioxide (CO_2) lasers emit light at a wavelength of a 10.600 nm, which is strongly absorbed by tissue water. The penetration depth is dependent upon the water content, independent of melanin and hemoglobin. It stimulates and promotes the regeneration of collagen fibers and restores hydration and elasticity in the vaginal mucosa[19]. Fractional erbium laser is a minimally invasive thermo-ablative fractional laser technique, which is applied to vaginal mucosa and is used in postmenopausal vulvar-vaginal atrophy, stress urinary incontinence, and vaginal tightening. With its wavelength of 2940 nm, it is close to the absorption peak of water. This laser has 10 to 15 times more affinity for water absorption compared with the fractional CO_2 laser. The photothermal effect of the laser beam heats the collagen in selected mucosal tissue leading to the contraction of collagen fibers and at the end shrinkage of tissue. It has minimal thermal damage to surrounding tissue so has milder postoperative discomfort and edema[20]. Laser vaginal rejuvenation was trademarked by Dr. David Matlock. It is performed with a 980 nm diode laser used as a cutting device, much like standard cautery, and not in the newer minimally invasive fractional manner now used by many laser companies to shrink the vaginal walls.

RF vaginal rejuvenation: this energy-based skin rejuvenation technology has been harnessed for rejuvenation of vaginal tissue to treat vulvovaginal laxity resulting from age or childbirth-related causes. Studies have shown that the use of RF for vulvovaginal laxity results in increased collagen and elastin formation[21]. Unlike laser-based treatments, it is not dependent on skin type and is even more effective in naturally moist tissue. This technique has been demonstrated to be especially well tolerated when using temperature-controlled RF. The target tissue temperature is 40-45 degrees Celsius, and thermistors enable monitoring and thermostating the temperature. This technique enables collagen denaturation and the healing process, supporting healthy tissue formation, which is the mechanism that provides tightening. Collagen fibers when heated contract and this causes the triple helix structure to fold, creating thicker and shorter collagen fibers, which are thought to be the mechanism of action of the immediate tissue tightening seen after these procedures (Figure 3a, 3b, 3c). The creation of new elastin, which is relatively unique to RF, may play a role in its effectiveness in treating vaginal laxity[22]. Additionally, it has been found that the increased local blood flow with this technology leads to decreased dryness due to vulvovaginal atrophy, resulting in improved sexual performance and satisfaction (Figure 4a, b, c)[23,24]. Researchers have also shown regression of stress urinary incontinence with tightening of the pubocervical fascia[25,26].

Vulvar lightening: this technique achieves whitening of a hyperpigmented vulvar appearance through chemical agents or the CO_2 fractional laser method. Avoiding rebound hypo-hyperpigmentation should be the prime objective[27]. Hyper and hypo-pigmentation can occur with the use of energy-based devices such as a CO_2 laser to lighten the area. The use of RF in an ablative manner can also result in both hypo or hyperpigmentation. Non-ablative RF avoids these pigment issues.

Platelet-rich plasma (PRP): autologous PRP was first reported in 1987 for open heart surgery[28]. Over 20 years it has been

studied in wound care, orthopedics, dental surgery, spine literature, and a variety of cosmetic surgery procedures. PRP contains high level of growth factors such as platelet-derived growth factor, transforming growth factor beta and epidermal growth factor. It is nonantigenic because it is autologous, and there have been no detected adverse effects[29]. It has been found that PRP injections are nonsurgical options for female sexual dysfunction, lack of lubrication, and stress urinary incontinence[30]. Some pilot studies have also shown that PRP has an effect in the treatment of lichen sclerosis[31,32]. O-Shot® is a PRP procedure that was trademarked by Charles Runels.

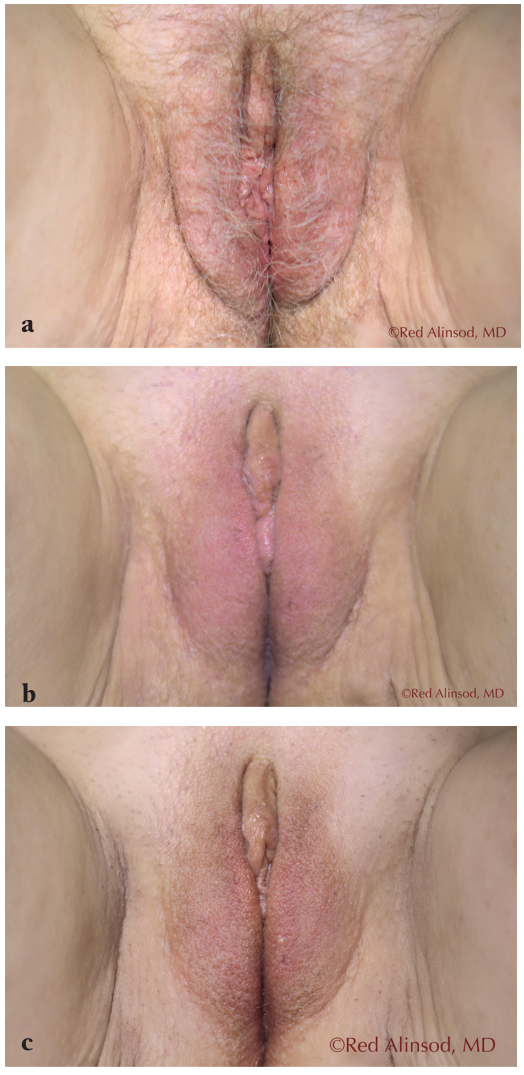

Figure 3. A 66-year-old female with severe genitourinary syndrome of menopause and labia majora laxity underwent monthly transcutaneous temperature-controlled radiofrequency vaginal rejuvenation x6 to achieve maximum labia majora shrinkage without surgery. Shown is the progressive tightening effects obtained over the 6-month period. Internal treatments were also done that enabled comfortable sex to be possible without the need for added lubricants a) before treatment, b) after 3 treatments, c) after 6 treatments

The combination of PRP with RF for lichen sclerosis has shown tremendous promise for long term symptoms relief. Studies in the United States with Dr. Runels and Dr. Alinsod are ongoing. augmentation: In 1950, German gynecologist Grafenberg[33] described an erotic zone on the anterior vaginal wall along the course of urethra. Since then, many articles have been published showing the existence of this zone and in 1981 this area was named as the G-spot by Addiego et al.[34] to honor Grafenberg[33]. Many authors accept this area as the responsible zone for vaginally-activated orgasm. The precise anatomy is not fully understood but can be defined as a neurovascular

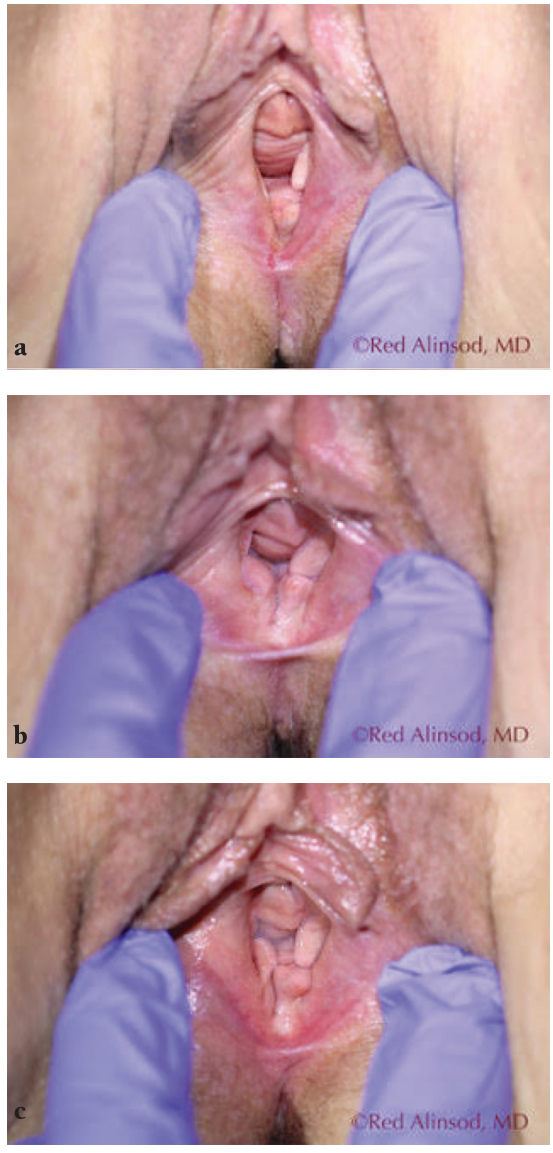

Figure 4. A 60-year-old female with severe genitourinary syndrome of menopause and tearing of perineum during intercourse. She underwent transcutaneous temperature-controlled radiofrequency monthly for 3 sessions and obtained excellent relief from the dryness and dyspareunia. Her incidentally-found mild cystocele was also reduced in size a) before treatment, b) after 2 treatments, c) after 3 treatments

complex[35,36]. G-spot augmentation with fillers such as collagen or autologous fat transplantation leads to bulking of this zone to the vaginal lumen and much more penetration during sexual intercourse[37].

Discussion

The perception of ideal external female genitalia or ideal labium appearance differs between countries[38]. The desired appearance according to countries affects the surgeons' techniques[8]. According to the American Society for Aesthetic Plastic Surgery, labiaplasty numbers increased 23% from 2015 to 2016[39]. Various techniques may be applied in labia minoraplasty according to the state, color change, and expectations of hypertrophy. Any desired reduction of labia can be provided with linear excisions but it cannot provide the retention of the natural look or coloration the patient currently has. The wedge resection and modifications of this technique may serve for patients who want more natural edges. When performed by surgeons trained and experienced in this field, these operations are demonstrated to improve the reliability of the procedures and the functional and aesthetic appearance[8].

Nonsurgical techniques like transcutaneous temperature-controlled and laser devices are also options for aesthetic genital interventions, especially for vulvovaginal laxity. Studies also showed some changes in vulvovaginal atrophy and stress urinary incontinence[40]. Patients with severe organ prolapse are not candidates for nonsurgical aesthetic techniques. Therefore, a careful examination should be performed to evaluate pelvic organ prolapse and several self-reported questionnaires can be used to assess the degree of symptoms[41]. There are some controversial issues as to which energy-based device is superior (laser or RF) or which type of laser device should be preferred. Unlike surgical techniques, non-surgical approaches need maintenance treatments, and treatment protocol differ according to the device. For the standardization of treatment modalities and an explanation of duration of efficacy we need further research.

Let us briefly examine how the world's leading gynecologic associations evaluate this issue. When the American Collages of Obstetricians and Gynecologist (ACOG) first addressed this issue in 2007, it opined that procedures such as vaginal rejuvenation designer vaginoplasty, and revirgination were seperate from procedures with non-aesthetic medical indications which had insufficient research so far[17]. However, after this committee's declaration, Ostrzenski.[42], in 2011, published extensive evidence-based work on the effectiveness and reliability of these procedures and concluded that the ACOG's 2007 recommendations did not comply with scientific norms and were not sufficiently transparent. Iglesia[43] in 2012, emphasized that the term "perfect vagina" represented a significant domain in the concept of women's beauty and that doctors must inform their patients about the complications and all the relevant details concerning this matter. In 2013, Canada's Society of Obstetricians and Gynaecologists's policy statement recommended that the medical, sexual, and gynecologic histories be reviewed with patients requesting genital cosmetic surgery. They also recommended that the patient be informed of the normal variations in genital appearance, the physiologic changes that develope with aging, and the unpredictability of changes that might occur during pregnancy and menopause[44]. In 2013, the Royal College of Obstetricians and Gynaecologists published ethical considerations in relation to female genital cosmetic surgery and recommended that "female genital cosmetic surgeries shouldn't be carried out before 18 years of age, the patient must be fully concerned about the procedures, and any advertising of these procedures conforms to good medical practice"[45]. In 2015, the International Federation of Gynecology and Obstetrics Committee for the Ethical Aspects of Human Reproduction published a report supporting that patients requesting cosmetic gynecologic procedures and surgeons must be aware of the differences between therapeutic surgical procedures and surgical procedures without medical indications, that normal anatomy and variations must be explained so that patients have a good understanding of them, that patients should be evaluated, especially for body dysmorphic disorder and other mental problems, and that the operating surgeons must have competent skills in this field[46].

In 2017 ACOG published a new committee statement recommending that in the case of requests for mammoplasty and labiaplasty, patients, especially adolescents, and their families be informed about normal variations and physical changes, that the patient's physical and emotional development had to be evaluated, and that consultation about non-surgical techniques should be provided[47].

According to the World Health Organization, the definition of female genital mutilation refers to all procedures involving partial or total removal of external genitalia[48]. It has no health benefit and it is a human rights violation. However, this description is totally different from female genital cosmetic surgery relating to genital destruction and the lack of patient consent in mutilation.

In conclusion, the lines between medically necessary operations such as vaginal/pelvic reconstructive surgery and elective surgeries such as vaginoplasty and labiaplasty are blurring and can now be performed at the same time. Both function and beauty are becoming addressed together and not separately. Minimal complication rates and maximum patient satisfaction can be achieved if experienced, trained physicians are involved. To achieve the best outcomes in both functionality and appearance, surgeons must inform patients about normal variations, perform psychological evaluations, and discuss realistic expectations. The technique of surgery to be used should be individualized with great consideration of the patient's preferred goals and with realistic expectations. The surgeon's training and skills and comfort level with the various techniques must be considered

fully. As is clearly seen, more academic training in this branch of gynecology must be given. Moreover, further studies are needed on the long-term efficacy, safety and reliability of non-surgical techniques, especially those that do not require hospitalization and can be performed in an office environment.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

Conflict of Interest: Red M. Alinsod is a consultant and speaker for Thermi, Cooper Surgical. Aylin Güneş has no conflict of interest.

Financial Disclosure: The authors have no financial support for this review.

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Review / Derleme

DOI: 10.4274/tjod.23911

Turk J Obstet Gynecol 2018;15:112-25

Oocyte *in vitro* maturation: A sytematic review

Oosit in vitro matürasyonu: Bir sistematik derleme

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Abstract

In vitro maturation (IVM) is one of the most controversial aspects of assisted reproductive technology. Although it has been studied extensively, it is still not a conventional treatment option and is accepted as an alternative treatment. However, studies have shown that IVM can be used in almost all areas where in vitro fertilization (IVF) is used and it has a strong place in fertility protection and Ovarian Hyperstimulation syndrome management. The aim of this systematic review was to address all aspects of the current knowledge of IVM treatment together with the evolution of IVM and IVF.
Keywords: In vitro maturation, clinical applications, laboratory procedure, pregnancy rate, fertility preservation

Öz

İn vitro matürasyon (IVM) yardımcı üreme teknolojilerinin en tartışmalı konularından biridir. Üzerinde çokça çalışma yapılmış olsa da halen klasik bir tedavi seçeneği değildir ve ancak alternatif tedavi olarak kabul edilmektedir. Oysa ki yapılan çalışmalarda, IVM'nin in vitro fertilizasyonun (IVF) kullanıldığı hemen tüm alanlarda kullanılabildiğini ve fertilite koruma ve Over Hiperstimülasyon sendromu yönetiminde önemli bir yer tuttuğu görülmektedir. Bu sistematik derlemenin amacı IVM ve IVF'nin evrimi ile birlikte güncel bilgiler ışığında tüm yönleriyle IVM tedavisini ele almaktır.
Anahtar Kelimeler: İn vitro matürasyon, klinik uygulamalar, laboratuvar prosedürleri, gebelik oranları, fertilitenin korunması

Introduction

The first in vitro fertilization (IVF) attempts were carried out with immature rabbit oocytes because in vivo matured oocyte retrieval was impossible in the 1930s[1-4]. Edwards.[5-7] conducted essential work on human oocyte *in vitro* maturation (IVM) during the 1960s and the first human IVF techniques were based on the use of IVM. IVM is the progenitor of current IVF treatment[8,9]. Mature oocyte collection from the preovulatory follicles in normal cycle women only became possible after the introduction of laparoscopy into gynecology practice in the 1970s[10]. With the advent of IVF and the successful delivery of Louise Brown, IVF following ovarian stimulation became the norm. Clomiphene citrate (CC), which was first marketed in the 1960s, was the first agent for ovarian stimulation. Later human menopausal gonadotropins (hMGs) were introduced into the IVF industry and either alone or in combination with CC, hMGs became the drug of choice in ovarian stimulation protocols[11-15]. Although hMGs increased the number of oocytes and the chance of pregnancy, they brought about Ovarian Hyperstimulation syndrome (OHSS), which could even be fatal for an otherwise healthy young woman[16]. This is one of the reasons why IVM regained attention in the 1990s as an alternative. Cha et al.[17] reported the first IVM birth from immature oocytes derived from oocyte donors. The first IVM baby from the mother's own immature oocytes was in 1994[18]. Worldwide, over 5000 IVM babies have since been born[19]. Natural-cycle IVF or mild-stimulation protocols have acceptable outcomes and some advantages over traditional IVF cycles, especially in poor responders[20]. IVM gained attention among fertility specialists for its safety, repeatability, cost effectiveness, and almost no risk of OHSS together with acceptable clinical pregnancy rates. Though the main indication was patients with polycystic ovaries (PCO), IVM has much wider indications including poor ovarian reserve and repeated IVF failures[21]. The term IVM refers to the maturation of the retrieved immature oocytes in the special culture environment. Exogenous gonadotropin stimulation for short courses seems to improve the ultrastructure of the

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Received/Geliş Tarihi: 08.04.2018 **Accepted/Kabul Tarihi:** 26.04.2018

oocytes expected to mature in *in vitro* conditions. In the strict definition, this is not IVM. However, this raises the question of whether a correct definition of IVM or the benefit patients gain from the treatment is more important. The kinetics of oocyte maturation and ultrastructural changes will be discussed in the following text. The nuclear and cytoplasmic maturation in *in vitro* conditions determine the competence of the oocytes and eventually embryo quality and clinical outcomes. Many authors recommend IVM as an alternative to traditional IVF, whereas others refuse to recommend IVM as a treatment option in the modern era[22,23]. However, contrary to the American Society for Reproductive Medicine opinion and the publication of De Zeigler, the accumulating data show that IVM is not an alternative, but should be accepted as a potential first-line treatment option[8,24]. There are many controversial issues that need to be clarified about IVM. Should we neglect IVM or adopt it as an important treatment option in IVF centers? The aim of this systematic review was to discuss all aspects of IVM in detail, from the definition to clinical outcomes.

Online literature search

The following keywords were used to search PubMed; *in vitro* oocyte maturation, clinical outcomes, indications, ultrastructural changes, fertility preservation. A total of 2753 papers were seen during PubMed and selective journal searches. A detailed search eliminated most of the papers and 456 papers from both PubMed and hand-searched assisted reproduction technology (ART) journals were re-checked.

Selection of eligible studies

One hundred forty-three full-text papers and 67 abstracts selected from PubMed and ART journals were selected for this systematic review.

Discussion

Terminology and description

Even the definition of human oocyte IVM is controversial. Dahan et al.[25] recently published a paper on the definition of IVM. Variations in treatment protocols, selection of patients, indications other than Polycystic Ovarian syndrome, number of embryos transferred, and cleavage stage embryo or blastocyst transfer are important factors. Thus, a clinical definition of IVM was introduced: The aspiration of small or intermediate-sized follicles for oocyte retrieval in the ovaries carrying follicles less than 13 mm in diameter. Short-course of gonadotropin use needs to be acknowledged but priming during monitorization may lead to the retrieval of mature oocytes together with immature oocytes. Thus, they recommended renaming the procedure as natural- cycle IVF or modified natural-cycle IVF with early triggering, combined with IVM[25]. We reported collecting only germinal vesicle (GV) oocytes in follicle-stimulating hormone (FSH)-human chorionic gonadotropin (hCG) primed IVM cycles of 165 patients. The reason is the largest size was less than 10 mm and hCG priming was given on the 8th day of the cycle[26]. The rationale for using gonadotropins prior to IVM was to trigger the developmental potential of the immature oocytes. The reason for using gonadotropins prior to IVM is to trigger the developmental potential of the immature oocytes and make them more compatible. Some authors may oppose the above mentioned definitions because they believe that IVM should never be primed with pharmacologic agents[27]. Edwards RG.[28] published a paper on the definition of IVF terminology and reported that IVM could be included in the list of definitions by using minimal/mild-stimulation IVM or natural-cycle IVM. There is another important issue that needs to be taken into consideration; one is that immature oocytes retrieved after long-term gonadotropin stimulation and culturing denuded immature oocytes from such conventional stimulated IVF patients is not in the vicinity of IVM and another is truncated IVF where just a single bolus of hCG or gonadotropin agonist is given for triggering without short FSH priming[29]. Mixed oocyte generation is seen in both these protocols which are matured *in vitro* and later fertilized[9]. Wang et al.[30] published an interesting article in which they studied 591 IVM cycles, 240 cycles of unstimulated IVM for PCO syndrome (PCOS), 153 cycles of IVM converted from stimulated IVF cycles for PCOS, 103 cycles of unstimulated IVM in non-PCOS patients, and 95 cycles of IVM converted from stimulated IVF cycles of non-PCOS cases were compared. They concluded that PCOS and IVM cycles rescued from stimulated cycles have higher implantation rates, better quality embryos, and acceptable clinical pregnancy rates. Also, IVM cycles converted from stimulated IVF cycles have lower abortion rates as compared with others. They also concluded that PCOS cases were more suitable for IVM treatment[30]. In an unpublished study 11 cases of stimulated IVF cycles that resisted gonadotropins were converted to rescue IVM and 6 patients had pregnancy and 4 had livebirths (Hatırnaz et al. article in review). The definitions proposed to date remain confusing because a definition should be short and simple, thus both definitions made by Dahan et al.[25] and Coticchio[27] need further evaluation.

Indications and clinical applications

IVM was first introduced in patients with PCOS and patients who had severe OHSS in their previous IVF treatments but the indications were expanded in recent years and in almost all areas of infertility; IVM can be adapted as an option. Potential indications of IVM are;

-PCOS
-PCO-like ovaries
-Normo-ovulatory patients
-Previous failed IVF attempts
-History of OHSS
-Oocyte maturation problems
-Patients with testicular sperm extraction (microdissection-TESE)
-Emergency oocyte retrieval due to malignancies (estrogen-sensitive tumors)

-Oocyte retrieval from ovarian tissue before vitrification
-Poor responders
-IVM for rescuing IVF cycles
-Resistant Ovary syndrome
-Recurrent implantation failure
-Preimplantation genetic diagnosis (PGD)/preimplantation genetic screening (PGS)

IVM was first introduced into clinical practice as an alternative treatment option in patients with PCOS[17]. Trounson et al.[18] reported that immature oocytes derived from IVM cycles retain their potential to grow under *in vitro* conditions and this can be a new therapy for infertile women with PCOS. From that time on, many studies focused on the use of IVM in other indications. Lindenberg[31] reported that IVM was also used in regularly menstruating women, low responders, and in patients with cancer. Papers on IVM before 2009 showed low implantation and pregnancy rates but after the publication of Pak et al.[32], the results were comparable with IVF. PCOS and OHSS are the main indications of IVM; however, IVM may also be used in cases of resistant ovary syndrome and fertility preservation as uncommon indications[33-35]. Child et al.[36] studied the impact of IVM on PCO, PCOS, and unstimulated normal ovaries and concluded that hCG priming in all three groups had similar high maturation, fertilization, and developmental potential. Seok et al.[37] studied the predictive role of anti-mullerian hormone (AMH) on the selection of IVM in patients with PCOS and concluded that AMH was a valuable factor in predicting clinical outcomes in such patients who preferred IVM as the treatment of choice. Gremeau et al.[38] studied 194 women with PCOS to evaluate the efficacy of IVM instead of conventional IVF and concluded that IVM was safer, simpler, and avoided the risks of OHSS secondary to IVF. Siristatidis et al.[39] reviewed IVM in patients with and without PCOS. In a meta-analysis of 11 studies, 268 patients with PCOS with 328 cycles and 110 patients with PCOS with 110 cycles were compared with 440 patients dendritic cells 1 and it was concluded that IVM was an effective treatment option when offered in subfertile women with PCOS[39]. Yoon et al.[40] studied pregnancy outcomes from IVM-derived oocytes in normoovulatory women and found a 17.6% pregnancy rate (9/51 embryo transfers). It was concluded that IVM in normoresponder cases might lead to successful pregnancies though the pregnancy rate was quite low[40]. Oocyte collection during the luteal phase opened a new dimension in ART and in patients with cancer wishing to preserve their fertility because luteal phase oocyte pick up is possible and efficient. Demirtas et al.[41] studied three women without male partners who were close to gonadotoxic chemotherapy due to malignancies. IVM oocytes were easily retrieved from luteal phase ovaries in these women and oocytes were vitrified for future use[41]. Fadini et al.[42] studied IVM in normoovulatory women and compared IVM with conventional IVF. They found that conventional IVF was superior to IVM in respect of the success rates and IVM could be an alternative intervention for some conditions[42]. Fadini et al.[43] also studied predictive factors in IVM and evaluated the role of body mass index, basal FSH and estradiol concentrations, antral follicle counts (AFC), endometrium thickness, and leading follicle size. Estradiol and FSH concentration and AFC were found to be predictive factors in the decision of whether to start IVM, and endometrial thickness and leading follicle were predictive factors for the timing of immature oocyte retrieval[43]. Tannus et al.[44] evaluated predictive factors in 159 IVM cases and concluded that duration of infertility, number of immature oocytes, embryo blastomere count, and embryo grade were predictive factors for live birth after IVM in patients with PCOS. Braga et al.[45] studied IVM in stimulated cycles in 440 poor responder patients. Immature oocytes associated with MII oocytes were divided into two groups and rescue spontaneous maturation oocyte-derived embryos were added to *in vivo* matured oocyte derived embryos in poor responder patients. They concluded that adding such embryos in poor responder patients had no impact on clinical outcomes, although it improved the number of embryos transferred and lowered the cancellation rates[45]. IVM may be a valuable option in patients who failed in conventional IVF. Gulekli et al.[46] studied 23 women who failed in conventional IVF and were transferred to IVM without ovarian stimulation. Only one pregnancy was obtained and that did not continue to birth, and the authors concluded that IVM might be a useful tool for failed conventional IVF[46]. As an uncommon indication, IVM may be an important optional choice in cases with oocyte maturation abnormalities. Hatırnaz and Hatırnaz[47] reported a patient with genuine Empty Follicle syndrome (EFS) who benefited from IVM oocytes retrieved from the patient and matured, but her partner was azoospermic and only a few sperms were derived from the microsurgical TESE (micro-TESE) procedure and one embryo on day 2 was transferred with a negative pregnancy test[47]. Hourvitz et al.[48] evaluated 7 patients with seven IVM cycles. Two of them were genuine EFS, one was PCOS with egg factor, two patients had repeated GV oocytes in their retrievals, and two had atretic oocytes. The patients with genuine EFS achieved pregnancy and the course of the other indications was not relevant. IVM may be the first choice in cases of genuine EFS[48]. Edwards reviewed new modalities that may replace routine IVF (IVM, natural-cycle IVF, minimal-stimulation IVF) and gave special attention to IVM. The author discussed the huge amount of information collected from the genetics and biochemistry of IVM oocytes and reviewed papers enlightening the future, and suggested that new follicular formation could be achieved from bone marrow or stem cells derived from a drop of blood both in children and adults[49]. There is a need for clarification of whether IVM should be evaluated strictly as a laboratory procedure alone or be accepted as part of the IVF treatment protocol. In the following section, types of ovarian stimulation and additional measures to optimize IVM outcomes from clinical site will be discussed.

Stimulation protocols and treatment modalities in *in vitro* maturation

The only difference of IVM from conventional IVF is the maturation of the oocytes under *in vitro* conditions. IVM is a laboratory term and obtaining immature oocytes is dependent

on certain clinical protocols, monitorization, and timing of oocyte retrieval, which is why IVM per se is not a treatment protocol, it is the laboratory part of a stimulation protocol or a stimulation cycle. Types of stimulations are listed below:

- Unstimulated IVM cycles without hCG priming
- FSH priming IVM cycles (75 IU/day for 3 days. Start at day 3)
- hCG priming IVM cycles (10.000 IU-20.000 IU IM when the endometrium reaches 8 mm)
- FSH and hCG priming IVM cycles (the combination of above protocols)
- Cycle independent IVM in cancer patients (random start or letrozole use)
- IVM cycles converted from conventional IVF (rescue procedure)
- Aromatase inhibitor use for ovarian stimulation in IVM (letrozole 2.5 mg twice daily start at day 3 for five days)
- Estrogen-suppressed in IVM (estradiol valearate started on day 3 of the cycle)

IVM is not something that promotes the initiation of processes that activate quiescent human oocytes. A fully-grown oocyte meiotic resumption is triggered by luteinizing hormone (LH) or by hCG administration before oocyte retrieval. Oocytes are covered by a thick layer of glycoprotein secreted by the oocyte itself called the zona pellucida. The zona is covered by specialized granulosa cells named corona cells, which make the cumulus oocyte complex (COCs) for the good nourishment of the oocytes. Oocyte maturation means the nuclear and cytoplasmic maturation processes, which should not necessarily happen at the same time. Nuclear maturation, the meiotic resumption process, transforms prophase oocytes to metaphase II (MII) oocytes[50]. Following meiotic resumption, the nuclear membrane dissolves, which is called GV breakdown (GVBD). For the developmental ability and fertilization of oocytes, cytoplasmic maturation seems to be as important as nuclear maturation[51]. The first live baby born from IVM, was the result of oocytes picked up at different stages of the menstrual cycle and derived from an unstimulated patient with PCOS[18]. Overcoming failure in IVM urged fertility specialists to transfer an average of 6.3 embryos[52]. Söderström-Anttila et al.[53] published an article on IVM from unstimulated patients with normal or PCOS ovaries and evaluated 239 cycles of immature oocyte retrievals without gonadotropin stimulation. Nine-hundred seventy-one immature oocytes from 122 IVF-IVM cycles were compared with 851 immature oocytes from 117 intracytoplasmic sperm injection (ICSI)-IVM cycles and found maturation and fertilization rates in IVF was 62.6% and 37.7% after IVF, whereas these rates were 53.9% and 69.3% after ICSI, respectively. The implantation rates and pregnancy rates were higher in the IVF-IVM group. They concluded that good pregnancy rates could be achieved in IVM cycles without gonadotropin stimulation and ICSI or IVF did not significantly change success rates[53]. Walls et al.[54]. studied IVF versus ICSI for fertilization in IVM cycles and compared 72 IVM-IVF cycles with 78 IVM-ICSI cycles and were able to follow up the embryos until the blastocyst stage. Blastocyst stage embryos were determined at higher rates in IVF-IVM than in ICSI-IVM, and the maturation rates were similar. It was concluded that IVM-IVF could be a viable method for obtaining good quality embryos and acceptable clinical pregnancy rates in PCOS-IVM cycles[54]. In order to increase the success rates in IVM cycles, priming with FSH or hCG has been recommended before oocyte retrieval[55-57]. Smith et al.[58] were first to describe the 10 mm cut-off value for the size of the leading follicle in order to obtain immature oocytes during retrieval. Management of OHSS is a major concern of IVM because FSH-priming stimulation of the ovaries will not trigger the secondary or tertiary cohorts in patients with PCOS who prefer IVM as the treatment of choice. In some circumstances, early follicular aspiration at follicular size <14 mm and *in vitro* oocyte maturation as an adjunct to matured oocytes may be preferred to overcome OHSS, which is known as rescue IVM (rescue IVM is also used for patients converted from conventional IVF due to difficulty in follicular growth in stimulated cycles)[59]. Fadini et al.[60] evaluated the effect of different modes of stimulation in IVM patients with normoovulatory ovaries and assessed 400 eligible women for the study. One hundred patients were treated without FSH priming, 100 were primed with hCG alone, 100 were primed with FSH, and finally, the last 100 patients were primed with both FSH and LH. The results of the study showed that FSH priming together with hCG priming had favorable outcomes compared with the other modalities. FSH priming or hCG priming alone makes no significant contribution to the clinical outcomes[60]. Contrarily, Mikkelsen and Lindenberg[56] stated that priming with FSH alone in IVM cycles might improve the maturational potential and implantation rates of the embryos derived from immature oocytes. Reinblatt et al.[61] studied the controversial issues in IVM and they categorized the problematic conditions under four areas, namely: 1) the benefits of gonadotropin use, 2) hCG priming and timing, 3) ideal endometrial preparation, and 4) luteal phase support. The authors concluded that prospective randomized and well-designed studies were necessary for both clinical applications and maturational processes of oocytes *in vitro*[61]. hCG priming may be substituted with recombinant LH in IVM cycles. Hreinsson et al.[62] used recombinant LH instead of hCG for priming in a randomized trial and found that rec LH was as efficient as hCG in promoting the maturation processes of immature oocytes *in vitro*. However, rec FSH use did not seem to be patient friendly because of the costs when compared with hCG. A French group reported their clinical outcomes in 33 PCOS patients with 45 IVM cycles in 2005 and obtained 26.2% pregnancy rate and concluded that hCG primed IVM might be an alternative to conventional IVF[63]. Farhi et al.[64] studied the use of OCP before IVM to decrease the laboratory overload related to IVM procedures and compared this approach with immediate-start IVM and concluded that pregnancy rates were

similar and programming of IVM cycles was possible. Vitek et al.[65] published an article on estrogen-suppressed IVM as a new and efficient IVM protocol and they evaluated the clinical and laboratory aspects of ES-IVM. This approach had similar outcomes to natural-cycle IVM or FSH-priming IVM and might eliminate the dependence on gonadotropins during IVM cycles[65]. This is one of the most controversial issues in IVM because early-onset estrogen, either alone as in this study, or in combination with FSH may suppress endogenous FSH and may have undesired effects on the oocyte maturation *in vitro*. Early-onset estrogen may influence synchronized GV oocyte retrievals, which is desired to overcome terminologic confusion[26]. Another interesting stimulation protocol for IVM is the use of letrozole for flare up of FSH for a short time while blocking the receptors reversibly. Rose studied letrozole use in IVM cycles and achieved successful pregnancies and ongoing pregnancies and deliveries[66]. Robertson et al.[67] studied letrozole use in IVM in 5 patients and achieved 3 pregnancies, 2 of which delivered healthy infants (Hatırnaz et al, article under evaluation). The use of letrozole is also very important in patients with cancer seeking fertility preservation and for those who need emergency IVM with a random-start protocol. Albuz et al.[68] studied cyclic AMP modulators added to pre IVM of bovine or mouse COCs and this was determined to increase COC cyclic AMP levels almost 100-fold. By this way, they simulated oocyte maturation physiology and named their method 'simulated physiological oocyte maturation' (SPOM). SPOM mimics the oocyte maturation *in vivo* and has benefits for oocyte IVM, which may be used in IVM protocols for better clinical outcomes[68]. Another important issue in IVM protocol is the timing and dosage of hCG priming and the time interval between hCG priming and oocyte retrieval. Endometrial thickness >8 mm together with a leading follicle <14 mm is preferred for the timing of hCG priming in IVM cycles[69]. A leading follicle size of <12 mm is accepted for obtaining GV oocytes in IVM cycles. The McGill group published an article related to the time interval after hCG and concluded that instead of 35 hours, a 38 hour interval until oocyte retrieval increased the chance of oocyte maturation and might influence the laboratory and clinical outcomes in cycles for IVM[70]. IVM has wide variety of indications and is open to novel treatment options. Combined with the advances in culture systems and more standardized protocols, IVM will take a greater place in IVF centers rather than as a neglected modality in the modern ART era.

Oocyte pick-up (retrieval)

Bovine studies have shown that the diameters of aspiration needles and vacuum aspiration pressure during immature oocyte pick-up (OPU) by the transvaginal route have significant impact on the morphology of COCs and this morphology is involved in the developmental capacity and competence of bovine oocytes[71].

The recommended pressure during human immature oocyte retrieval varies from 56 mm hemoglobin (Hg) to 180 mm Hg and aspiration needle diameter ranges from 16-20 gauge[18,29,72-74]. The cross-sectional area of a 17-gauge needle is 3.57 times wider than that of a 20-gauge needle. The recommended vacuum pressure for IVM OPU is 80-100 mm Hg. Lower pressure aspiration together with a 20-gauge aspiration needle may improve the developmental competence of oocytes derived from IVM cycles[75]. Techniques of OPU, total time for OPU, the use of a flushing medium during immature OPU, and the temperature regulations in the aspiration pump system are also important factors for the developmental potential and cytoplasmic maturation of oocytes *in vitro*.

Laboratory procedures

Mammalian oocytes are dependent on the follicular environment for proper maturation. Oocytes and follicles have symbiosis-like interrelations because the follicle loses its competence when the oocyte ovulates from the follicle. In the meantime, oocyte development and meiotic resumption takes place in the follicular milieu after the LH peak. Removal of immature oocytes from the follicles blocks the completion of maturation processes and no well-developed culture environment will be sufficient to perfectly nourish and mature the oocytes derived from IVM. There have been more animal studies on IVM than human studies and information derived from large animal studies may illuminate the path of human oocyte IVM. The follicular conditions at the time of oocyte recovery and the oocyte chromatin distribution may have a great impact on the clinical outcomes in human IVM studies[76]. Chromatin condensation begins when the preantral follicles become early antral follicles in mammalian oocytes. The oocyte itself can determine its own fate. Oocyte-derived growth differentiation factor (GDF) 9 and bone morphogenetic protein (BMP) 15 have regulatory roles on the proliferation of granulosa cells, thus the oocyte has great potential to arrange its own environment for nuclear and cytoplasmic maturation[77]. The use of human and animal model studies together with advancing technologies may open new areas of IVM use in ART practice. It is important to know that the origin of the germ-line stem cells does not have to be ovarian but may be derived from bone marrow or peripheral blood, as reported by Edwards[78]. In an experimental study, the oocytes in the ovaries of mice were destroyed chemically and the oocytes removed and later injected with bone marrow cells and the chemically-depleted ovary resumed small antral follicle formations[79]. Adding matrix metallopeptidase to heat-stressed bovine oocytes during IVM has not been shown to improve the *in vitro* oocyte growth and even resulted in a detrimental effect[80]. The development of culture systems for animals provides valuable information to develop culture media for human oocyte IVM. For the monitoring of oocyte competence and maturation *in vitro*, a three-step culture system has been developed but the processes *in vivo* is accelerated in culture systems with optimizations for

oocyte competence[81]. It is important to understand the cellular and molecular events that occur in the follicular environment coordinate both oocyte and somatic cell development. This course of events eventually raises the quality of the consistency of culture media used for *in vitro* oocyte maturation in humans. Oocyte-secreted factors, mainly GDF 9 and BMP 15, regulate the COCs and cumulus cell function and follicular granulosa cell functions *in vivo* and have a great impact on the quality of oocytes[82,83]. Understanding the intrafollicular environment and developmental mechanisms of oocytes *in vitro* will clarify the apoptotic processes that result in oocyte atresia or EFS, which may be overcome with IVM procedures[47,48]. The quality and consistency of the culture media used for *in vitro* oocyte maturation remains a dilemma in the era of extensive gonadotropin use. OHSS, which is a complication of IVF drugs, is a life-threatening condition and the risk is almost zero in IVM cycles, although it has been attempted to resolve the complication related to the drugs used in IVF with another drug instead of IVM. This is an important matter and few reports support the idea that IVM is useless in modern ART. The number of patients preferring IVM as the treatment of choice has reduced and financial resources for culture media development are restricted. Although there are a few publications opposing IVM[22,23], there is an increasing number of publications supporting IVM in both clinical and ultrastructural points. Access to IVM culture media whenever required is not easy due to the reduced demand of centers for IVM, but in reality, all IVF laboratories need to learn and include IVM in their routine practice, rather than neglect it. Therefore, follicular fluids (FF) are co-cultured with culture media together with cumulus corona complex or hCG, and FSH in predetermined doses is added to the culture environment to support the development of immature oocytes. Such additions may influence the nuclear maturation but have no effect on cytoplasmic maturation[84,85]. Early embryonic development has many interactions and complex intracellular and extracellular correspondence. Studies have reported a number of physiologic changes in culture environments for remarkable oocyte growth and maturation *in vitro* although fully imitating *in vivo* conditions seems impossible. Adding FSH, LH, and human serum albumin to the culture medium of IVM means that there is much to do to develop well-standardized culture media[86]. IVM of human oocytes is a necessity for different ovarian pathologies. Investigating human oocytes for maturation problems has ethical issues and limitations. The major determinant of embryonic development is the quality of the oocyte, which is the GV oocyte in IVM. Two GV oocyte types have been recorded; one is the surrounded nucleolus (SN) and the other is the non-SN (NSN). SN GV oocytes have great developmental competence compared with NSN, which can be determined by heterochromatin staining around the nucleolus. Finding SN oocytes may improve the clinical outcomes of IVM cycles. Besides the low developmental potential or arrest of the NSN oocytes, there is also a relationship with the reduced expression of ribosomal proteins, especially cytoplasmic lattices, which can be used as morphologic markers[87]. Culture media used in in vitro oocyte maturation is the cornerstone of IVM cycles, but trials on the efficiency of the culture media and studies to develop much better cultures are unfortunately limited. Two IVM culture media are currently used in IVM practice and in one study immature oocytes derived from C-section patients were shared in Medicult and Sage culture media and the results were compared. The authors concluded that there was no difference between the media in respect of fertilization, cleavage, and blastocyst formation rates[88]. Filali et al.[89] reported a retrospective study on the efficacy of (two culture media-199 and IVM-Medicult). Both media showed similar outcomes concerning oocyte maturation, fertilization, embryonic development, and clinical pregnancy rates[89]. For immature oocytes derived from stimulated IVF patients, commercially available IVM culture systems may not be enough for the maturation of GV and MI oocytes[90]. IVM culture media need some additions for oocyte IVM , mainly autologous serum of the patient and FSH and LH added as stock solutions. Lin et al.[91] and Goud et al.[92] studied adding growth factors [Epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), activin, transforming growth factor-beta or granulosa cell co-culture) in standard IVM medium for immature mouse oocytes and concluded that the addition of growth factors did not improve the clinical outcomes[91,92]. Contrary to the above publication, Jahromi et al.[93] reported that the addition of granulosa cells in human oocyte IVM medium as a co-culture might improve the developmental competence and maturation of oocytes. Optimized IVM procedures should be studied extensively in well-designed human clinical trials instead of animal models. Animal studies have shown correct imprinted DNA methylation establishment in oocytes but such a conclusion cannot be reached in human IVM. Epigenetic analyses from babies born from IVM treatment will clarify the epigenetic safety of the procedure in humans[94]. hCG priming IVM-derived oocytes are correlated with their maturation time and those oocytes reached MII in a shorter time than other GV oocytes, and had higher developmental competence and better success rates. This study showed that *in vitro* culture systems favored nuclear maturation in human oocytes but cytoplasmic competence needed further studies to be improved[95]. IVM may have some undesired effects on the chromosomal alignments and spindle structure of the immature oocytes within the culture environment. In a study, 304 oocytes from 101 women and spindle configurations were detected by using PolScope and evaluated with immunohistochemical staining for alpha tubulin and chromatin. The authors concluded that supplementing the culture media might improve the maturation rate but not indicate the presence of spindles and chromosomal alignment[96]. The culture media and the plastic materials used in laboratories are important in oocyte IVM. Bisphenol-A (BPA)

may, in a dose-related manner, decrease the MII transformation of GV oocytes and increase the rate of oocyte degeneration in the laboratory. Chromosomal alignment and bipolar spindle formation decreases as MII oocytes come into contact with higher BPA concentrations[97]. Elizur et al.[98] evaluated 15 women to evaluate the corpus luteum formation potential of minimum-sized follicles during hCG priming IVM cycles and measured estrogen and progesterone levels 5-7 days after oocyte retrieval and antral follicles were recorded. They discovered a new cohort of follicles during their study, which supported the studies of Baerwald et al.[99]. The occurrence of fertilization failure in IVM and IVF may be attributed to the short interval of hCG triggering and ICSI and the lengthening of this interval may overcome the risk of FF in IVM cycles[100]. Nuclear and cytoplasmic maturity are sequential but independent processes in the maturation of oocytes and culture-related conditions may alter the course of maturation processes, mainly cytoplasmic maturation, and eventually may result in abnormalities or in increased aneuploidy rates. For this reason, oocyte-donor immature oocyte-derived embryos from 11 patients were compared with embryos for PGD-sex selection. The study revealed that preimplantation genetics or prenatal chromosome studies should be recommended to patients preferring IVM as the treatment of choice[101]. An important study on the chromosomal abnormality rates in IVM cycles was conducted in McGill University and 6 IVM cycles and 30 IVF cycles for fluorescence *in situ* hybridization (FISH) analysis were compared and the results showed that the aneuploidy rates in both groups were similar. The aneuploidy rate in IVM patients with a maturation interval of 48 hours had significantly higher aneuploidy rates compared with 24-hour interval matured oocytes[102]. Real-time continuous embryonic follow-up by time-lapse incubation in IVM cycles of patients with PCOS was studied revealing an increase in the early embryonic arrest but the morphokinetic changes during embryonic development were not altered. Multinucleation at the two-cell and four-cell stage and uneven blastomeres at the two-cell stage were higher in PCOS-IVM cycles. Embryo arrest at day 3 to day 4 transition was seen to be higher in PCOS-IVM patients[103]. Improved culture systems and optimized protocols in PCO-IVM and PCOS-IVM patients led to good blastocyst formation, improved implantation and pregnancy rates in single-embryo transfer. One hundred to 150 IU FSH started at day 3 of the cycle for 3 days plus hCG priming when the leading follicle reached 10-12 mm and estrogen added to the protocol on the day of oocyte retrieval when the endometrium was 6 mm. Eight hundred forty-four oocytes from 66 patients were collected and 588 oocytes were matured in vitro, 420 oocytes were fertilized, and 175 embryos reached blastocyst stage. Sixty-two good grade blastocyst embryos were transferred as single-embryo transfer and 28 live births were achieved. The authors concluded that optimized IVM protocols might result in good laboratory and clinical outcomes[104]. Early estrogenic supplementation on day 3 in both FSH and LH priming IVM cycles of 159 PCOS patients for optimum clinical outcomes revealed that homogenous immature oocyte retrievals were achieved in such cycles and single-embryo transfer was a feasible option in IVM cycles and prevented multifetal gestations[26].

Ultrastructural changes in *in vitro* matured oocytes

EGF and IGF-1 were found to augment the spontaneous maturation of oocytes *in vitro*[105,106]. After publications of mouse FF-meiosis activating sterol (MAS), a trial was held to induce *in vitro* oocyte maturation by the addition of MAS in human oocytes, and it was seen that FF-MAS positively influenced human oocyte morphokinetics and nuclear maturation[107]. The supplementation of forklosin, an adenylate cyclase activator, and cilostamide, a phosphodiesterase inhibitors specific inhibitor, were added to the maturation culture media of immature oocytes and these agents were found to be influential in maturation processes and meiotic resumption[108]. Different maturation stages of oocytes have different morphokinetic characteristics during IVM. Cumulus cells surrounding abnormal oocytes carry high apoptotic potential, and abnormal oocytes had degenerated cellular structures in ultrastructural studies. Electron microscopic (EM) studies revealed that microvilli were rare around GV oocytes, the zona pellucida is thin and loose outside. Following GVBD, microvilli surrounding MI oocytes are common and large in size. EM of MII oocytes has shown mitochondrial degeneration and diminished cristae in the mitochondria (M) together with an increased amount of apoptotic activity in cumulus cells[109]. In one study, 204 immature oocytes were evaluated for ultrastructural changes and 101 GV oocytes were compared with 103 MI oocytes. Maturation rates were higher in MI oocytes and immature oocytes showed large M-vesicle complexes VCs. Transmission EM findings of MII oocytes were dense fibrillary zona pellucida, uniform perivitelline space, a continuous oolemma and regularly distributed microvilli. The ultrastructure of GV oocytes from IVM have similarities with MII oocytes, but the most pathognomonic finding of GV oocytes is numerous large M-VCs. The authors concluded that immature human oocytes from IVM at different stages of development showed minimal cytoplasmic alterations in EM studies[110]. *In vitro* matured oocytes were evaluated ultrastructurally before and after vitrification and vitrified thawed oocytes were compared with previtrified oocytes concerning ultrastructural changes including M-smooth endoplasmic reticulum M-vs, the number of cortical granules, the integrity of oolemma and microvilli, vacuolization, and surrounding zona. The authors concluded that the ultrastructure of matured oocytes from IVM cycles had similar cytoarchitecture in both vitrified-warmed and previtrified oocytes in EM studies[111]. Dal Canto et al.[112] studied the morphokinetics of embryos derived from *in vitro* matured oocytes from hCG-primed IVM cycles. Oocytes derived from 8-12 mm follicles were categorized according to the cumulus expansion and expanded cumulus oocytes were

evaluated as MII and incubated for 6 hours, whereas others were accepted as GV oocytes and incubated for 30 hours. The authors concluded that morphokinetic behavior of both mature and GV oocytes from IVM cycles were comparable and it was suggested that only minimal differences were present in GV and MII oocytes from IVM cycles[112]. Embryos derived from GV oocyte maturation in stimulated IVF cycles had a high arrest rates and multinucleation rates but meiotic resumption happened normally. The aneuploidy rates were higher among these embryos determined by blastomere biopsy and FISH analysis[113]. The impact of oocyte IVM on the distribution of M was studied in China. Two hundred eighty-four immature oocytes derived from stimulated IVF cycles were evaluated and 140 were fixed. Other oocytes were prepared for IVM before the fixation process. All 21 oocytes matured *in vivo* were fixed directly and stained to visualize the M. The mitochondrial distribution was observed using confocal microscopy. Three types of mitochondrial distribution pattern were observed; peripheral, semiperipheral, and evenly diffused. Pre IVM oocytes showed high peripheral distribution and post IVM oocytes showed evenly-diffused M. The M of *in vivo* mature oocytes showed more central localization. The authors concluded that this might explain the diminished developmental competence of the IVM oocytes[114]. Similar outcomes were reported by Takahashi et al.[115]. Melatonin could induce meiotic maturation in bovine and porcine immature oocytes. This investigation illuminated the study of low- concentration melatonin use in human oocytes and 1 nM dose was found to be optimal for human GV and MI oocytes and to have a positive influence on nuclear maturation during rescue IVM[116]. Why do *in vitro* matured oocyte-derived embryos have low implantation potential? IVM and *in vivo*-matured (IVO) oocytes derived from pseudopregnant mice were compared, and after 5 days, the implanted blastocysts were removed from the mouse uterine horns and the uterine horns were analyzed for mRNAs, some growth factors, progesterone receptors, and homeobox A10. The maturation rates of GV oocytes were high but the implantation and fertilization rates were quite low compared with IVO and all mRNAs derived from IVM derived embryo horns were significantly diminished compared with IVO. The authors concluded that implantation-related mRNAs were diminished and thus the developmental competence of IVM-derived embryos was lower than IVO[117]. The presence of double-strand DNA breaks were found in immature oocytes and DNA integrity was an integral part of meiotic resumption in IVM[118]. Reduced oxygen concentrations in the laboratory environment for the development of immature oocytes and embryos is an important factor that can be measured by the expression patterns of glucose metabolism genes[119]. PGS or PGD for some genetic problems may be applied in IVM embryos that have reached the blastocyst stage and the first healthy baby born from PGD for chromosomal translocation was reported in an IVM cycle[120]. Practicing PGD in IVM may eliminate developmentally-incompetent and aneuploid embryos, which eventually may improve the implantation and pregnancy rates with healthy deliveries.

Vitrification in *in vitro* maturation

Cryopreservation of *in vitro* matured oocytes is a new dimension in assisted reproduction. Cryoprotectants may have oocyte meiotic spindle damage and may alter mitochondrial function and integrity during vitrification. *In vitro*-matured oocytes from donors have shown that spindle and chromosomes were not affected by vitrification solutions[121]. There are a few alternative kits for vitrification. An Italian group studied the use of Cryotop vitrification in IVM GV oocytes and compared them with GV oocytes and MII oocytes from fresh cycles. Light microscopy and phase contrast microscopy results showed no significant changes in the oolemma and cytoplasm. The ultrastructural features of GV oocytes were preserved during Cryotop vitrification and the authors concluded that the GV stage seemed more suitable for vitrification than MII oocytes[122]. To determine the strength of GV oocytes, 184 immature oocytes were divided into two groups and 100 MII oocytes were vitrified for 24-48 hours after IVM and another 84 GV oocytes were vitrified directly and *in vitro* matured after thawing. The survival of the thawed oocytes and thawed and *in vitro*-matured oocytes were similar but the maturation rate was higher in the group that was matured first and vitrified later. The authors concluded that IVM maturation was more efficient when performed before GV oocyte vitrification[123]. Day 3 vitrified-warmed embryos from *in vitro* matured oocytes have the same potential to reach the blastocyst stage compared with fresh embryos. However, vitrified oocyte-derived embryonic transition to cleavage stage embryos, namely day 3, was low, and the authors concluded that *in vitro*-matured oocyte-derived embryonic development before day 3 was diminished but after genomic activation; embryos that pass beyond day 3 to day 5 were not affected by vitrification[124]. During maturation, oocytes use Ca^{2+} for many physiologic processes and in mouse studies, vitirification solutions including dimethyl sulfoxide caused temporary rises in Ca^{2+} concentrations. CP also increases intracellular Ca^{2+} concentrations. The same physiologic principles may be used in human oocytes and GV oocytes were randomized into five groups. G1; GV oocytes matured by IVM, G2; vitrified at GV stage, G3; GV oocytes matured by IVM and then vitrified, G4; human oocyte IVM through the intracellular oscillations, and G5; GV oocytes exposed to ionomycin and IVM until MII. The authors concluded that osmotic shock from the vitrification solutions might have influenced the maturation capacity of IVM oocytes[125]. Vitrification under 196 °C does not mean that the cryoenvironment is aseptic; therefore, protection of embryos or oocytes is mandatory and a carrier and storage system that separates the gametes and embryos from others is recommended for laboratories[126].

In vitro maturationin fertility preservation

Cancer and fertility preservation in young people is an important medical concern and needs to have strategies developed. For fertility preservation in patients with cancer, a multidisciplinary team including specialists in gynecological oncology, general surgery, oncologists, assisted reproductive technology team, clinical embryologists, and genetic specialists is necessary. Ovarian tissue freezing, immature oocyte harvesting from ovarian tissue specimens, and oocyte maturation *in vitro* become key troubleshooting factors in patients with cancer, especially if urgent cancer treatment is ahead[127]. Improved cancer treatment outcomes in young males and females have led physicians to fertility preservation measures to avoid major sequelae of rigorous cancer treatments. Sperm and oocyte cryopreservation, testicular and ovarian tissue freezing, and cryopreservation of embryos derived from IVM oocytes are great challenges for fertility preservation. However, some technical, legal, and ethical concerns have yet to be clarified such as informed consent from patients who are minors, legal parentage, and medical negligence[128]. Immediate IVM was preferred in a 27-year-old woman who presented for fertility preservation prior to pelvic radiotherapy and had a laparoscopic radical hysterectomy for cervical carcinoma. Due to the risk of vaginal dissemination risk of the disease, the ovaries were removed and oocytes were removed from the ovariectomy specimen by *ex vivo* aspiration and then 22 oocytes were matured *in vitro* and 15 oocytes were matured to MII oocytes in 24 hours and vitrified. The remaining oocytes were followed up for a further 24 hours and 7 more oocytes reached MII phase and were vitrified as a second round. This is a good modality of fertility preservation in patients with cancer who are short of time because of radiotherapy and chemotherapy[129]. A Canadian group evaluated 41 women with cancer who had undergone IVF treatment and compared them with 48 women as a control group. They found that younger women with malignancies maintained their ovarian reserve, responded to gonadotropins well, and oocyte retrieval and maturation rates were unchanged, but the same recommendations cannot be made for spermatogenesis[130]. The same group studied patients with breast cancer (n=87), hematologic malignancies (n=16), and gynecologic or abdominal malignancies (n=9) who were treated with IVM and compared them with 79 infertile controls. Ovarian reserve and maturity rates were found to be similar in malignancies other than breast cancer, and patients with breast cancer treated with IVM had fewer oocytes retrieved[131]. Fadini et al.[132] reported a patient with ovarian cancer who was treated conservatively and oocytes were recovered from antral follicles, matured *in vitro*, and then the developed embryo was vitrified and later warmed; a 2-cell embryo was transferred but failed to achieve pregnancy. Ovarian stimulation in patients with breast cancer is almost impossible, thus unstimulated IVM has become a valuable option[133]. Oktay et al.[134] studied the use of IVM as a complementary treatment for 32 patients with breast cancer and 464 oocytes were retrieved, of which 274 were matured. Immature oocytes were matured in IVM culture media and a total of 399 oocytes were matured; fertilization by IVM was higher than spontaneous maturation. Thus, IVM is a useful strategy for obtaining mature oocytes for fertility preservation[134]. IVM has found a new area of use in oophorectomy specimens and immature oocytes are recovered and cryopreserved, and babies delivered from those oocytes have been reported. An average 14 oocytes from each of 34 patients were retrieved with an overall maturation in vitro rate of 36%. Although most patients preferred oocyte vitrification, 8 patients preferred embryo freezing and 1 patient preferred to have embryo transfer and ongoing pregnancy was achieved after warmed embryo transfer[135,136]. Oocytes can be retrieved from postpubertal female children at risk of premature ovarian failure due to Turner syndrome or cancer; retrieved oocytes have been matured and cryopreserved from girls who accepted fertility preservation and all the required procedures were well tolerated[137]. Fertility preservation is not restricted to patients cancer, it can also be used in other medical conditions[138]. Indications of fertility preservation other than cancer are listed below:

- Premature ovarian failure
- Chromosomal and genetic abnormalities (Turner syndrome, 47, XXX, Fragile XGALT enzyme or FSH receptor mutation)
- Autoimmune diseases (thyroid, polyglandular, multiple endocrine)
- Environmental factors (malaria, varicella, Shigella may cause POF)
- Surgical menopause (benign ovarian disease, prophylactic oophorectomy)
- Cytotoxic agents for hematologic and autoimmune diseases
- Postponed fertility.

Obstetric and perinatal outcomes in *in vitro* maturationin

The first baby born from immature human oocytes harvested from unstimulated ovaries from a gonadectomy patient and used in donor oocyte program was reported by Trounson et al.[18]. Cha et al.[52] also reported clinical pregnancies and deliveries from IVM oocyte-derived embryos in 64 patients with PCOS. Twenty-three of 85 ET cycles of 64 patients with PCOS (27% pregnancy rate) resulted in pregnancy. Seventeen patients delivered 20 normal-appearing infants[53]. Söderström-Anttila et al.[139] studied the obstetric and perinatal outcomes of children born from IVM cycles. IVM born babies were followed up carefully because this ART technique has rarely been applied. Forty-three women who delivered 40 singleton babies and three sets of twins were followed up for 2 years and the results showed that both perinatal-obstetric outcomes and development of the children in the two years' follow-up were normal[139]. Early studies of IVM revealed embryo transfers on day 2-3, but recently, blastocyst transfers achieved and pregnancy outcomes related to blastocyst transfer have been published. From 106 hCG-priming IVM cycles of 82 patients,

blastocyst transfer was achieved and the implantation rate was 26.8% and the pregnancy rate was 51.9%, which is favorable. Fifty-five cycles with blastocyst transfer resulted in clinical pregnancies. Forty-three women delivered 33 female and 24 male babies. The results were compared with cleavage-stage embryo transfer and the clinical pregnancy rate was found significantly higher in blastocyst transfer[140]. However, the rate of miscarriage among IVM cycles was higher as compared with IVF and ICSI cycles. This may be attributed to PCOS itself rather than IVM treatment[141]. In a study held in Italy, 196 babies (153 singletons and 43 twins) born from IVM treatments were evaluated for obstetric and perinatal outcomes. Among the twin pregnancies, one fetus was diagnosed with Down syndrome and aborted. Obstetric and perinatal outcomes were compared in detail with ICSI outcomes of the control group and the authors concluded that the outcomes were comparable including the major and minor abnormalities[142]. An interesting study from Japan reported imprinting genes and epigenetic factors related to IVM babies. EM studies were performed on different stages of IVM oocytes for oxygen consumption and blood from umbilical cords of babies born from IVM treatments. Neither oxygen consumption of oocytes nor imprinting gene defects from babies delivered were found in IVM cycles and the authors concluded that IVM was not related to imprinting gene disorders[143]. Another group from Germany studied the DNA methylation pattern (epigenetic role) in children born from IVM-ICSI and compared 11 IVM-ICSI babies with 19 controls. Chorionic villus sampling and cord-blood sampling were used for the evaluation and their results showed that no significant differences were found in either group. With regards epigenetics, the frequency of such imprinting defects or expressions is quite low[144].

Concluding remarks

- The course of IVM is progressing slowly and has a long way to go.
- IVM seems to remain an alternative option until standardized terminology and stimulation protocols are in place.
- The best IVM program may be FSH-hCG priming, yielding 100% GV oocytes with favorable clinical outcomes.
- Nuclear maturation and cytoplasmic maturation are not concordant and cytoplasmic maturation needs to be investigated extensively.
- Embryonic arrest seems more prevalent within the first three days but embryos beyond day 3 are more competent.
- Epigenetic changes in IVM are not significant and not more than changes in conventional IVF.
- IVM can be applied to all indications in which conventional IVF is applied.
- Enrichment of culture media for cytoplasmic maturity may increase the clinical outcomes in IVM cycles.
- For fertility preservation, IVM seems a remarkable option.
- PGS/PGD can be easily performed from the embryos of IVM cycles.
- Embryos derived from IVM oocytes are more susceptible to cryopreservation than GV oocytes.

The enrichment of culture media, standardization of the stimulation protocols and management of cytoplasmic maturity are strongly recommended for improved IVM cycles. Future fertility preservation and young age malignancies draw attention on IVM and as a conclusion, increasing the experience of IVM is recommended for all IVF laboratories, instead of neglecting it.

Acknowledgement

The authors would like to thank.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ş.H., E.H., Design: B.A., M.H.D., S.L.T., Data Collection or Processing: J.T., S.T., Ş.H., E.H., Analysis or Interpretation: B.A., S.L.T., Literature Search: Ş.H., E.H., Writing: Ş.H., E.H., B.A., S.T., J.T., M.H.D., S.L.T.

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

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

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Short Communition / Kısa Yorum

DOI: 10.4274/tjod.23245

Turk J Obstet Gynecol 2018;15:126-9

Anatomic structure of the internal iliac artery and its educative dissection for peripartum and pelvic hemorrhage

Anatomik açıdan arteria iliaca interna ve peripartum ve pelvik kanama için eğitici disseksiyonu

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Abstract

The abdominal aorta is divided into two parts (right and left) at the level of the fourth-fifth lumbar vertebra and called the common iliac artery. Anterior to the sacroiliac joint, common iliac arteries are divided into external and internal iliac arteries. The external iliac artery supplies the lower limb, and the internal iliac artery is the major vascular supply of the pelvis. Internal iliac artery is divided into anterior and posterior trunk. The anterior trunk supplies the pelvis, visceral organs, and the posterior trunk supplies pelvic parietal structures. The broad ligament envelopes the uterus anteriorly and posteriorly with its sheets and continues as the pelvic peritoneum at the lateral side of the pelvic wall. After cutting the pelvic peritoneum, the retroperitoneal area is visualized and the internal iliac artery with other great vessels of the abdomen can be noted.

Keywords: Internal iliac artery, dissection, postpartum, hemorrhage, obstetrics

Öz

Arteria abdominalis lumbar 4. vertebra hizasında sağ ve sol arteria iliaca communis olarak iki dala ayrılır. Articulatio sacroiliaca ön yüzünde ise arteria iliaca communis, arteria iliaca eksterna ve interna olarak dallanır. Arteria iliaca eksterna alt ekstremitenin arteryal kanlanmasından sorumlu iken arteria iliaca interna pelvisin arteryal kan akımını sağlar. Anterior (ön) ve posterior (arka) bölüm olarak ikiye ayrılan arteria iliaca internanın; ön bölümünden çıkan dallar temelde visseral organları beslerken, arka bölümden çıkan dallar pelvik duvar ve gluteal yapılar gibi parietal yapıları besler. Uterusu önden ve arkadan saran ligamentum latum uteri pelvik yan duvarda abdominal yüzeyleri saracak olan pelvik periton olarak devam eder. Dolayısıyla pelvisin lateral duvarında periton kesildiğinde retroperitoneal bölge açılır ve arteria iliaca interna ve batın içerisindeki diğer büyük damarların retroperitoneal olarak seyrettiği görülür.

Anahtar Kelimeler: İnternal iliak arter, disseksiyon, postpartum, kanama, obstetrik

Introduction

The abdominal aorta is divided into two parts (right and left) at the level of the fourth-fifth lumbar vertebra and called the common iliac artery. Anterior to the sacroiliac joint, the common iliac arteries are divided into external and internal iliac arteries. The external iliac artery supplies the lower limb and internal iliac artery is the major vascular supply of the pelvis[(1)]. The internal iliac artery is one of the two divided parts of the common iliac artery, it passes medially over the pelvic brim and runs downward to the pelvic cavity. At the upper margin of the greater sciatic foramen, it is divided anteriorly and posteriorly. The ureter stands at the medial aspect of internal iliac artery, the pararectal space is noted between the ureter and the internal iliac artery. It is the main blood supply to the pelvic organs, gluteal muscles, and perineum, with the anterior (visceral supply) and posterior (parietal supply) trunks. There is an important and increased potential of anatomic variations for the internal iliac artery, especially for the end branches, so the pelvic surgeon should be careful during the dissection of this area. Figure 1 and Figure 2 demonstrates the branches of the internal iliac artery.

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Received/Geliş Tarihi: 09.04.2018 **Accepted/Kabul Tarihi:** 08.05.2018

Anterior trunk of internal iliac artery runs anteriorly along the lateral pelvic wall and supplies most of the pelvic viscera. The arteries are the umbilical (obliterated), uterine, superior vesical, vaginal, obturator, middle rectal, internal pudendal, and inferior gluteal arteries[2]. The posterior trunk of the internal iliac artery runs posteriorly to the pelvic wall and gluteal region[3]. The arteries are as follows: the iliol umbar, which anastomoses with the superior gluteal and circumflex iliac arteries; the lateral sacral, which anastomoses with the median sacral artery; and the superior gluteal artery (Figure 3, lateral view of right internal iliac artery after dissection), which anastomoses with the lateral sacral, inferior gluteal and internal pudendal arteries (Figure 4).

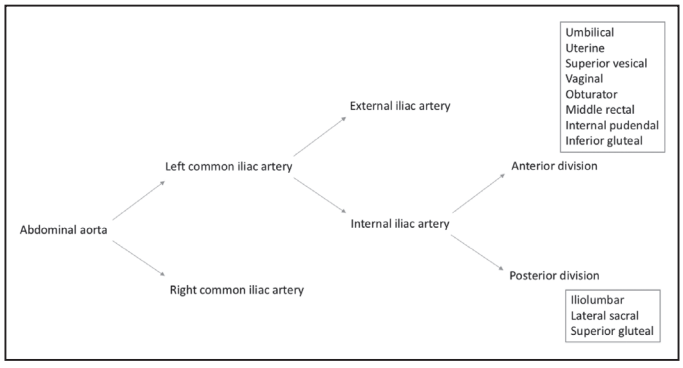

Figure 1. Branches of internal iliac artery

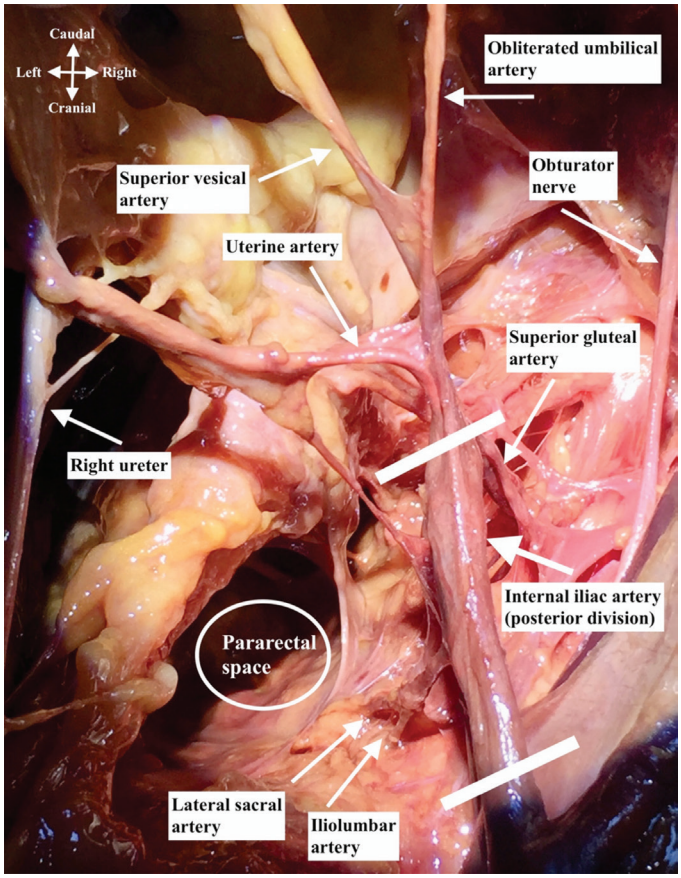

Figure 2. Right internal iliac artery dissection, superior view

The superior gluteal artery is the main part and continuation of posterior trunk and sometimes it may arise directly from the internal iliac artery. It runs between the lumbosacral trunk and first sacral nerve.

Materials and Methods

This cadaveric dissection was performed at the "Pelvic Reconstructive and Functional Urology Surgery Cadaveric Workshop"; an advanced masterclass course on anatomy and surgery by using fresh frozen cadavers on 22-23 April 2017 at Bahçeşehir University Faculty of Medicine, Prof. Rhoton Anatomy Laboratory, İstanbul/Turkey.

Arteries of anterior division

Obliterated umbilical artery

It is the blind end of the internal iliac artery (Figure 2, superior view of right internal iliac artery after dissection) and an important anatomic landmark to identify the uterine artery, especially during laparoscopic procedures. Moreover, it is in close relation with paravesical space and it divides the

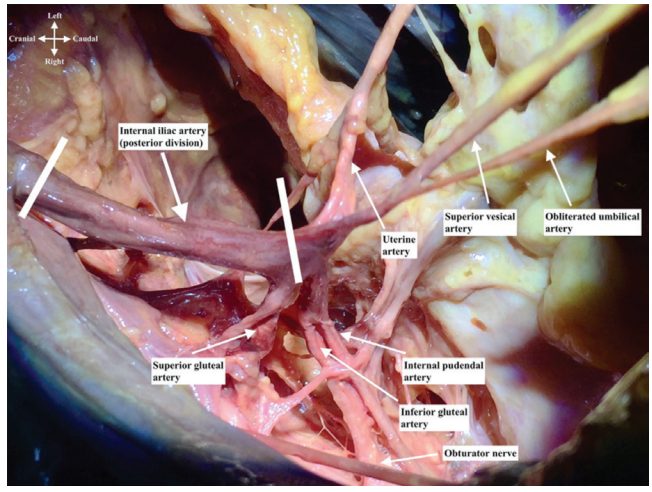

Figure 3. Right internal iliac artery dissection, lateral view

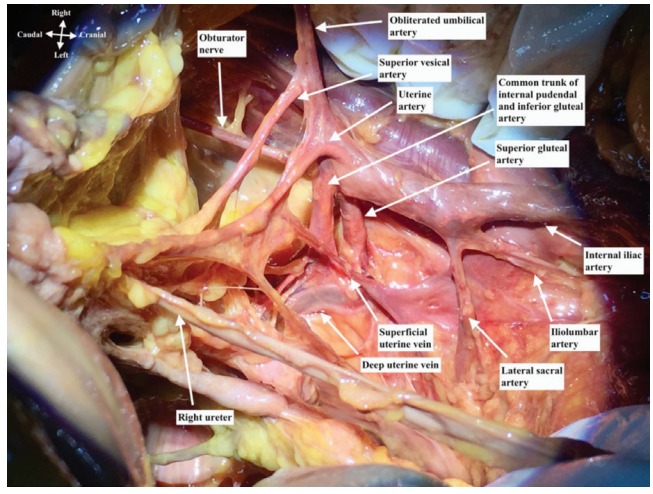

Figure 4. Right internal iliac artery dissection, medial view

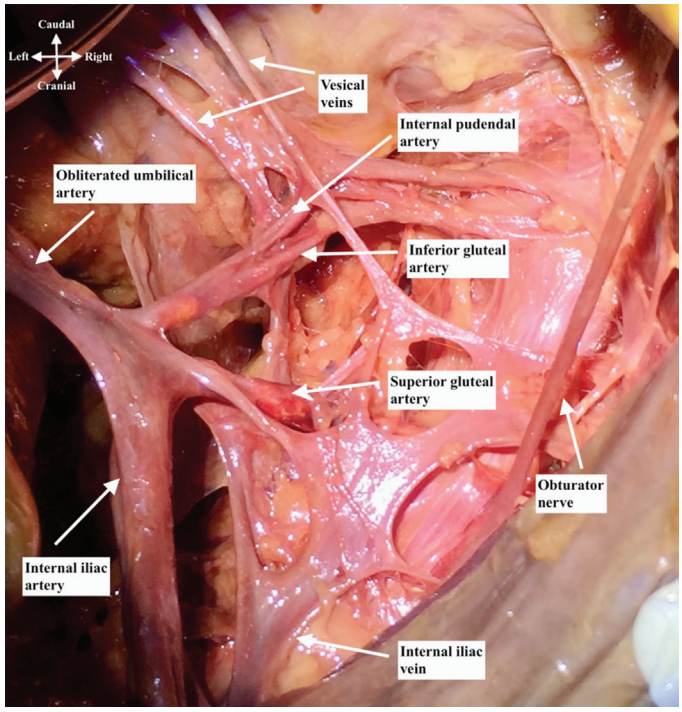

Figure 5. Right internal iliac artery dissection over the internal iliac vein, lateral view

paravesical space into two parts. When traction is applied where it attaches to the anterior abdominal wall, it may indicate the uterine artery. The superior vesical artery arises from the proximal part of the umbilical artery, which supplies the upper portion of the bladder. The inferior vesical artery supplies the base of the bladder; however, it is not possible to detect it during every dissection every dissection. It is also called the vaginal artery. The umbilical artery becomes the medial umbilical ligament when it reaches the anterior abdominal wall.

Uterine artery

The uterine artery arises from the anterior division of the internal iliac artery and goes antero-medially to the lateral part of uterine cervix below the isthmic part of uterus, where it crosses the ureter superiorly (Figure 4, medial view of right internal iliac artery after dissection). It begins between the origins of the obturator and umbilical arteries; however, sometimes it may have a common trunk with the superior vesical artery. The uterine artery runs between the anterior and posterior layer of broad ligament, above the cardinal ligament. It anastomoses with the ovarian artery and also gives a vaginal branch.

Clinical tip: how to identify the uterine artery?

Downward dissection from the obliterated umbilical artery is one way to identify the uterine artery. Another method is to follow the ureter to the point where it crosses under the uterine artery. Moreover, after opening the retroperitoneal region on the pelvic sidewall, antero-medial dissection of internal iliac artery may show the uterine artery.

Obturator artery

The obturator artery runs antero-infero-laterally on the obturator fascia and exits from the pelvis through the obturator foramen. The origin of the obturator artery varies; however, it mostly arises near the origin of the umbilical artery. It goes just under the obturator nerve and anastomoses with the inferior epigastric artery or external iliac artery via the pubic branch (corona mortis); in the pelvis, the obturator artery gives rise to the pubic branch it leaves the pelvis from the obturator foramen[4].

Clinical tip: obturator artery

An aberrant (accessory obturator artery), a variation, may arise from the external iliac artery and runs to the obturator foramen.

Clinical tip: corona mortis

Posterior to the superior pubic ramus at the superior part of the paravesical space, a vascular connection between the external iliac artery or inferior epigastric artery and the obturator artery can be detected. These vascular connections are mostly venous; however, the surgeon should be careful, especially for arterial anastomoses[5].

Internal pudendal artery

This artery runs infero-laterally, anterior to the piriformis muscle and sacral plexus (Figure 5). It passes through the greater sciatic foramen along the posterior aspect of the ischial spine close to the sacrospinous ligament and enters the ischio-anal fossa through the lesser sciatic foramen. Injury of the internal pudendal artery could be managed by compression on the ischiorectal/ischioanal fossa. The internal pudendal artery gives rise to the dorsal artery of clitoris.

Inferior gluteal artery

This passes between the sacral nerves, usually S1 and S2, sometimes S2 and S3 and leaves the pelvis through the greater sciatic foramen below the piriformis muscle (Figure 5). The inferior gluteal artery is also a danger zone during sacrospinous ligament fixation[6].

Superior vesical artery

It goes antero-inferiorly from the anterior division of internal iliac artery (sometimes it may arise from the same origin with uterine artery or from the obliterated umbilical artery) and supplies the distal end of the ureter and bladder.

How to dissect the internal iliac artery?

- The broad ligament envelopes the uterus anteriorly and posteriorly with its sheets and continues as the pelvic peritoneum at the lateral side of the pelvic wall.
- After cutting the pelvic peritoneum, the retroperitoneal area is visualized, the incision is extended to the paracolic area parallel to the infundibulopelvic ligament.
- The posterior sheet of the broad ligament is pulled medially, where the ureter and infundibulopelvic ligament lie.
- The common iliac artery is identified over the sacroiliac joint (the ureter crosses over it).

- When the adipose and lymphatic tissue is retracted softly over the vessels, the internal iliac artery is identified over the pelvic brim.
- The internal iliac artery first goes downwards, then straight to the anterior abdominal wall where it ends as the umbilical artery.
- When the adipose and lymphatic tissue is dissected over the internal iliac artery, the posterior division is identified at the first 3-4 cm, after this part, the anterior division starts and ligation of internal iliac artery could be performed at this point.
- For intractable pelvic hemorrhage the ligation of the internal iliac artery should be performed at the beginning of anterior division.
- Care should be taken with the external iliac vein, where it lies at the lateral part of the artery.

Ethics

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.S., E.H., İ.T., M.Y., Concept: İ.S., E.H., İ.T., Design: İ.S., E.H., M.Y., Data Collection or Processing: İ.S., İ.T., Analysis or Interpretation: İ.S., E.H., M.Y., Literature Search: İ.S., Writing: İ.S., E.H., İ.T.

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

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

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Case Report / Olgu Sunumu

DOI: 10.4274/tjod.82642

Turk J Obstet Gynecol 2018;15:130-2

External iliac artery thrombosis after hypogastric artery ligation and pelvic packing for placenta previa percreta

Plasenta previa perkreata yönetimi için hipogastrik arter ligasyonu ve pelvik kompres sonrası eksternal iliak arter trombozu

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Abstract

Placenta previa percreta is a serious pregnancy condition that may cause massive bleeding. Life-threatening hemorrhage is commonly managed via cesarean hysterectomy or vascular ligations in order to preserve fertility. We present a case of bilateral external iliac artery thrombosis after pelvic compression and uterine devascularization due to placenta previa percreta. The patient had cesarean section due to ultrasonography and magnetic resonance imaging-diagnosed placenta previa percreta, and stated that she preferred a conservative approach rather than hysterectomy in a case of massive bleeding. Spontaneous hemorrhage was recognized during the operation. Pelvic compression and bilateral uterine and internal iliac artery ligations were performed. The left external iliac artery was accidentally held and bonded as the left internal iliac artery, which was turned loose within a minute after distinguishing the vessels. Emergency angiography that was applied because of patient's leg pain showed bilateral external iliac artery thrombosis. Angioplasty was performed by a cardiologist for bilateral occlusions. Placenta invasion abnormalities may be managed by pelvic compression or vascular ligations, which have their own serious complications that the surgeon must manage immediately.
Keywords: Arterial ligation, pelvic packing, thrombosis, placenta previa, placenta percreta

Öz

Plasenta previa perkreata aşırı kanama ile sonuçlanabilen ciddi bir gebelik durumudur. Yaşamı tehdit eden kanamalar genellikle sezaryen histerektomi veya fertilite korumaya yönelik bir yöntem olarak damar ligasyonları ile kontrol edilir. Plasenta previa perkreata sebebiyle uterus devaskülarizasyonu ve pelvik kompresi takiben gelişen bilateral eksternal iliak arter trombozu olgusu sunulmaktadır. Hasta ultrason ve manyetik rezonans görüntüleme ile tanısı konan plasenta previa perkreata nedeniyle sezaryen operasyonuna alındı, ameliyat öncesinde masif kanama durumunda uterus koruyucu cerrahi tercih ettiğini belirtti. Operasyon sırasında spontan kanama fark edildi. Bilateral uterin ve hipogastrik arterlerin bağlanması ardından pelvik kompres uygulandı. Sol eksternal iliak arter sol hipogastrik arter ile karıştırılarak bağlandı, bir dakika içerisinde fark edilerek sütür açıldı. Post operatif dönemde hastanın bacak ağrısı tarif etmesi üzerine acil anjiyografi çekildiğinde bilateral eksternal iliak arter trombozu fark edildi. Bilateral tıkanıklıklar için kardiyoloji uzmanı tarafından anjiyoplasti uygulandı. Plasenta invazyon anormallikleri cerrahın acil müdahalesini gerektiren komplikasyonlarla sonuçlanabilecek olan pelvik kompres veya damar ligasyonları ile opere edilebilir.
Anahtar Kelimeler: Arter ligasyonu, pelvik kompres, tromboz, plasenta previa, plasenta perkreata

Introduction

Placenta percreta is a serious condition which is managed via pelvic artery ligation, preventive embolization of specific arteries, placental retention, or cesarean hysterectomy[(1)]. Pelvic packing may be considered to support either artery ligation or hysterectomy to reduce an uncontrolled hemorrhage[(2)]. Here we present a case of bilateral external iliac artery thrombosis following ligation of the bilateral hypogastric arteries and a transiently tied left external iliac artery, which underwent immediate angiography and thrombolysis.

Case Report

A thirty-three-year-old woman was admitted to our clinic at the 37th week of her gestation for delivery with a history of gravidity six, parity two, and abortion three. She had one previous

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Received/Geliş Tarihi: 14.03.2018 **Accepted/Kabul Tarihi:** 08.05.2018

cesarean section delivery. Her preoperative hemoglobin was 10.8 g/dL, prothrombin time (PT) 10.5 seconds, activated partial thromboplastin time (aPTT) 29 seconds, international normalized ratio 2.3, and platelet count 385x10^9/L. Transabdominal sonography and magnetic resonance imaging displayed total placenta previa, with myometrial invasion to the urinary bladder at the anterior wall of uterus (Figure 1, 2). The patient stated preoperatively that she preferred a conservative approach rather than hysterectomy in case of massive bleeding. After cesarean delivery of a transverse baby through a Pfannenstiel incision and removal of the placenta, a 5-6 cm area of tissue loss was detected at the anterior wall of the uterus and bleeding occurred from the cervix and posterior wall of the bladder. Pelvic packing was applied on the pelvic vessels for 20 minutes, the bleeding sites were sutured with 1.0 polyglactine sutures, and Sengstaken-Blakemore balloon catheter was placed in the uterus before suturing. A stomach balloon was filled with 250 mL saline and an esophageal balloon was filled with 400 mL saline to provide compression on the lower uterine isthmic and cervical bleeds, nevertheless, bleeding continued. Bilateral uterine and hypogastric artery ligations were planned due to hemorrhage. The left external iliac artery was accidentally held and bonded as the left hypogastric artery, which was released within a minute after distinguishing the vessels. Following this mistake, the uterine and hypogastric arteries were ligated on both sides. The patient lost about 2000 cc of blood due to the intraoperative hemorrhage as measured by adding 1650 cc blood in the aspirator and counted gauzes. She received erythrocyte suspension (3 units preoperatively and 4 unites postoperatively) and 3 packs of fresh frozen plasma. The patient had no findings of hypotension or shock at any time. The patient reported severe pain in both legs in the recovery room; it was observed that left dorsalis pedis and femoral artery pulses were absent. Doppler sonography showed a distinct stricture and triphasic flow loss on the left femoral artery. Diagnostic angiography was performed by a cardiologist. After a 6-F introducer sheath was inserted, it was confirmed that both external iliac arteries were occluded (Figure 3). Intravenous heparin (100 IU/kg) was administered afterwards. A 6-F left internal mammary artery catheter was used with 0.035 hydrophilic guide wires to cross the occlusion. Angioplasty

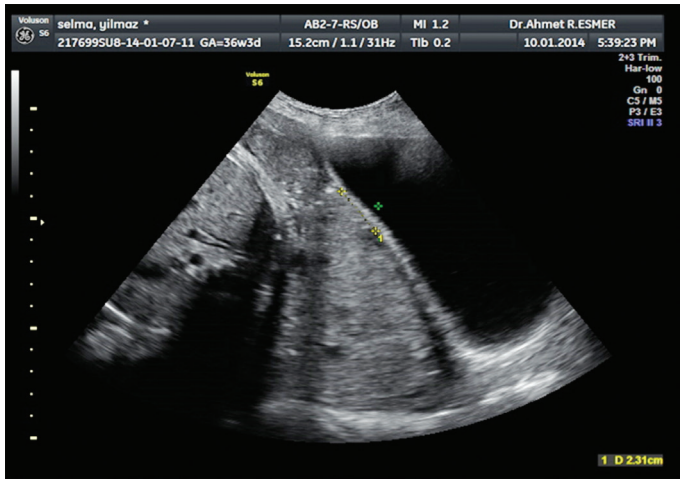

Figure 1. Transabdominal ultrasonographic view of total placenta previa

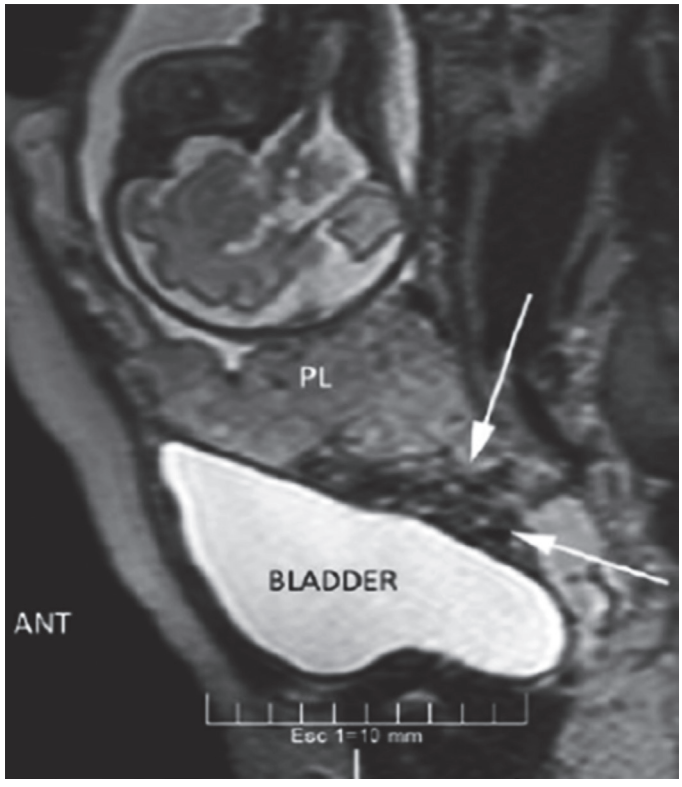

Figure 2. Magnetic resonance imaging of placenta percreta bladder invasion

ANT: Anterior, PL: Placenta

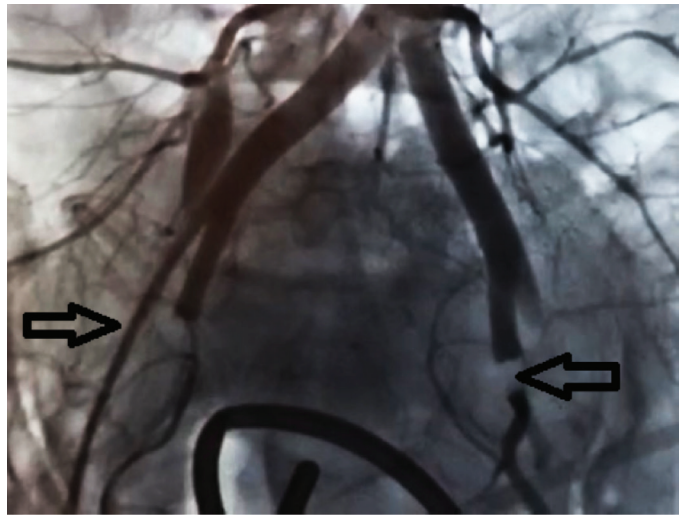

Figure 3. Right and left external iliac arteries. Left external iliac artery failed to show contrast during angiography because of thrombosis, and right external iliac artery was not filled properly with contrast due to thrombosis

was performed first to the right and then contralaterally to the left external iliac artery with a standard balloon (8x80 mm). A completion angiogram concluded the procedure. The femoral access site managed manually with digital pressure. The balloon catheter was removed after 30 hours. The patient and her child were discharged on the 4th postoperative day with no further events. Informed consent was obtained from the patient.

Discussion

There are mainly two different approaches to placenta implantation abnormalities to prevent excessive blood loss: cesarean hysterectomy or uterus protective techniques. The conservative approach is mainly considered when the patient prefers to spare her uterus for future fertility. Currently, placental invasion abnormalities are managed via radical or staged cesarean hysterectomy, vascular ligations and balloon embolization, placental retention, complex compression hemostasis for which pelvic packing is combined with uterine balloon placement, and partial hysterectomy for focal placental invasions[3,4]. The surgeon should be alert to complications of these management techniques. It is reported that common iliac artery thrombosis and acute limb ischemia, unilateral arterial rupture, bilateral pseudoaneurysms, and diminished bilateral leg blood supply due to thrombus and unilateral external iliac artery thrombosis are complications following bilateral hypogastric artery ligation[5]. In addition, one case of unilateral external iliac artery thrombosis due to placental retention has been reported[6]. Common iliac artery embolization is applied in some cases to prevent excessive hemorrhage due to an abnormally invasive placenta. This method can result in unilateral external iliac artery thromboembolisms and unilateral dorsalis pedis artery thromboembolisms[7]. Pelvic packing is an approach to reduce the hemorrhage and helpful in the management of abnormally invasive placenta[2]. Although this technique is useful to decrease the bleeding, it may cause significant complications. It is reported that deep vein thrombosis is a possible outcome of pelvic packing due to either pelvic fracture or excessive uterine bleeding[8]. There are no reports of arterial thrombosis following pelvic packing. In addition, it is important to note that the swabs used for pelvic packing are generally removed after 36-48 hours via relaparotomy, whereas it was applied for only twenty minutes and removed intraoperatively in our case. According to the patient's preference, cesarean hysterectomy was avoided in this case. Pelvic packing is selected in conservative techniques and supported by pelvic packing. Since placental retention carries the risk of postoperative hemorrhage and infection, this technique was not preferred for management of the case[3]. In our case, it was possible to anticipate left external iliac artery thrombosis because of accidentally holding the external iliac artery during the operation. However, thromboses were observed postoperatively in both the left and right external iliac arteries. This study is the first case showing bilateral external iliac artery thrombosis after the internal iliac artery ligation, and arterial thrombosis after pelvic packing about which the surgeon must suspect and manage immediately via angiography.

Ethics

Informed Consent: Taken from the patient.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.R.E., E.Ç., B.T., Concept: E.Ç., B.T., Design: E.Ç., A.R.E., Data Collection or Processing: R.A., E.Ç., Analysis or Interpretation: R.A., Literature Search: R.A., E.Ç., Writing: R.A.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study received no financial support.

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Letter to the Editor / Editöre Mektup

DOI: 10.4274/tjod.22309

Turk J Obstet Gynecol 2018;15:133-4

Diagnostic sign of intra uterine extra amniotic adhesions with 4D ultrasonography: Sheet on string

İntra uterin ekstra amniyotik adhezyonların 4D ultrasonografi ile tanısı: İpteki çarşaf

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Keywords

Extra amniotic adhesions, 4D ultrasonography, sheet on string

Anahtar Kelimeler

Ekstra amniyotik adhezyonlar, 4D ultrasonografi, ipteki çarşaf

Dear editor;

Intra uterine adhesions seen in pregnancy, which were defined in 1894 by Joseph G. Asherman, are divided into two groups as intra amniotic and extra amniotic adhesions. Intra uterine intra amniotic adhesions, which are also known as intra amniotic bands, are easily detectable in first and second trimester ultrasonographic examinations; therefore, close follow-up could provide an appropriate approach. Also intrauterine extra amniotic adhesions are a causative factor of infertility, early pregnancy loss, preterm delivery, cesarean section due to malpresentation, placental invasion abnormalities, and intra uterine fetal death[1,2]. There are no ultrasonographic diagnostic signs for intrauterine extra-amniotic adhesions in the literature, and the presumption of negative pregnancy outcomes and following up these patients is difficult for obstetricians. The aim of this study was to provide a handy method to distinguish intrauterine extra- amniotic adhesions for pregnancy outcomes and postpartum follow-up. Based on second trimester detailed ultrasonography outcomes, twenty-four patients were identified as having intrauterine extra amniotic adhesions through 4D ultrasonographic investigations. Sixteen patients had cesarean deliveries; the indications were previous cesarean section for ten, presentation abnormality for four, and placentation abnormality for two of the pregnant women. The intrauterine extra amniotic adhesions were verified during cesarean operations. Eight of the pregnant women had vaginal deliveries and six months after the delivery, adhesions were verified via hysteroscopic imaging. The locations of the adhesions were observed as following: fifteen of the women who had cesarean section had isthmic adhesions (93.75%), and one had cornual adhesion (6.25%). All eight women who had vaginal deliveries had adhesions in the uterine cornu (100%). In conclusion, intrauterine extra-amniotic adhesions can be identified by a specific ultrasonographic appearance known as "sheet on string," because it looks like a sheet spread out on a string (Figure 1, 2). Intra uterine adhesions that have not been identified in the preconception period but are important for pregnancy follow-up can be determined using 4D ultrasonography during anomaly screening with the advantage of amniotic fluid's image quality increasing effect.

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Received/Geliş Tarihi: 18.03.2018 **Accepted/Kabul Tarihi:** 20.03.2018

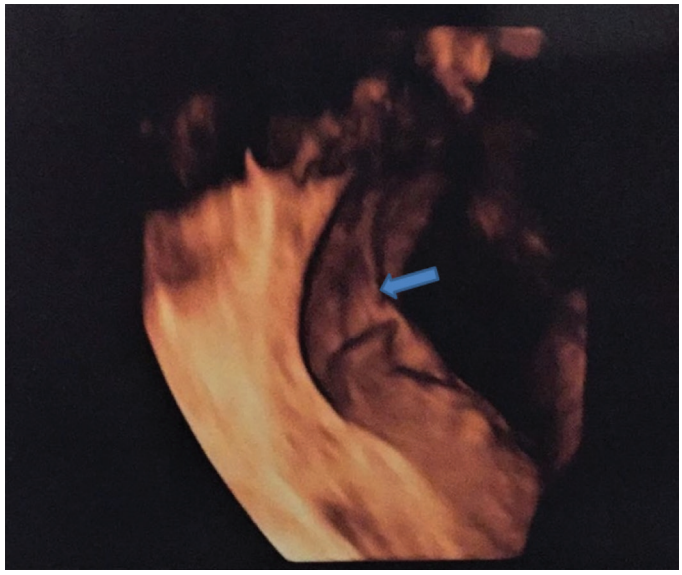

Figure 1. Intrauterine extra-amniotic adhesion demonstrated using 4D ultrasonography; "sheet on a string" appearance

Ethics

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ç., Concept: E.Ç., Design: E.Ç., Data Collection or Processing: R.A.B., R.A., Analysis or Interpretation: R.A.B., R.A., Literature Search: R.A.B., R.A., Writing: R.A.

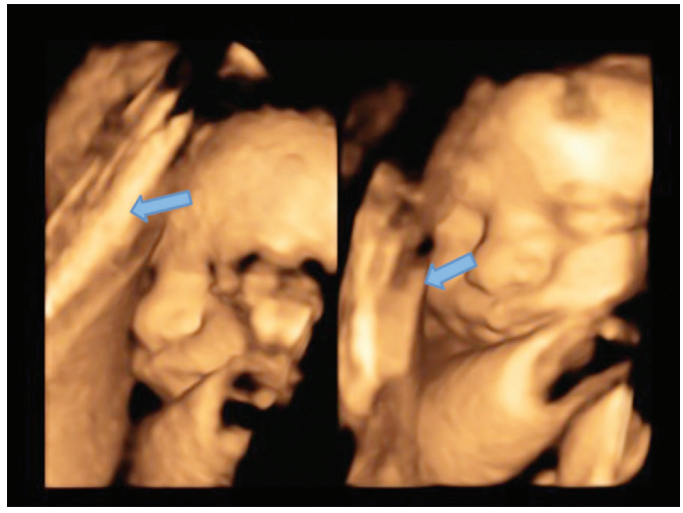

Figure 2. Intrauterine extra-amniotic adhesion demonstrated using 4D ultrasonography; "sheet on a string" appearance

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

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

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