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galenos yayınevi

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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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appropriately investigated and resolved. The statement about the authors' contributions should be placed in the cover letter. All persons who contributed to the work, but not sufficiently to be authors, must be acknowledged.

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Cover letter to the editors addressing the following points:

· The authors' intent to submit solely to Turkish Journal of Obstetrics and Gynecology.

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· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

· Informed consent.

· Any explanations related to reporting guidelines.

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CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/),

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).

CARE guidelines are designed to increase the accuracy, transparency, and usefulness of case reports. (Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D; the CARE Group. The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development.) (http://www.care-statement.org/)

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All manuscripts must be submitted as Microsoft Word (.doc or .docx) files. All manuscript pages (including references, tables, and figure legends) must be double-spaced. Use a standard, 12-point typeface such as Times New Roman. Top, bottom, and side margins should be set at 1 inch. Authors must include the following in the manuscript file:

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A separate title page should list;

-The manuscript title, which should contain no more than a total of 100 characters (counting letters and spaces) and should not be declarative; do not use abbreviations or commercial names in the title.

- 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.

Several types of articles can be submitted for publication in Turkish Journal of Obstetrics and Gynecology: Original research, case reports, systematic reviews, current commentaries, procedures and instruments, and letters. Stated word counts and page limits were shown in Table 1. Copyright transfer forms, the cover letter, and figures do not contribute to the page limits.

Table 1. Manuscript length at a glance

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.

Conflict of Interest

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:

Introduction, Case Report, Discussion and References.

References

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.

Use references found published in peer-reviewed publications that are generally accessible. Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references. Papers accepted by peer-reviewed publications but not yet published ("in press") are not acceptable as references.

Journal titles should conform to the abbreviations used in "Cumulated Index Medicus".

Examples

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Tables should be included in the main document after the reference list. Color figures or gray-scale images must be at minimum 300 DPI resolutions. Figures should be submitted in "*.tiff", "*.jpg" or "*.pdf" format and should not be embedded in the main document. Tables and figures consecutively in the order they are referred to within the main text. Each table must have a title indicating the purpose or content of the table. Do not use internal horizontal and vertical rules. Place explanatory matter in footnotes, not in the heading. Explain all abbreviations used in each table in footnotes. Each figure must have an accompanying descriptive legend defining abbreviations or symbols found in the figure. If photographs of people are used, the subjects must be unidentifiable and the subjects must have provided written permission to use the photograph. There is no charge for color illustrations.

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Units of measurement should be in Systéme International (SI) units. Abbreviations should be avoided in the title. Use only standard abbreviations. If abbreviations are used in the text, they should be defined in the text when first used.

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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 Collagues,

I am glad that our journal to reach this satisfactory level with your great contributions. I am also aware of the fact that we have to move forward in order to further progress.

I herein thank in the first place to the editor of the journal and also those who work hard to make the journal worthy of the doctors countrywide.

I especially thank the obstetrics and gynecology community for your support for the TJOD congress which will be held between May 9-13, 2018. We are preparing for our congress with a great excitement and enthusiasm.

I will be pleased to meet and recognize a few new academician collague as it occurs in every congress. I think this congress would be an unique opportunity for our young collagues to express and show themselves, and to enhance their scientific knowledge as well.

In addition, there would be a marvellous ambiance for the young collagues with the most experienced and knowledgeable colleagues of all the major branches of our specialty, to inform all the women about the reproductive health.

I look forward to seeing each and every one of our precious collague in our congress which will be held between May 9-13, 2018.

Best regards,

Ateş Karateke, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

After our inclusion in the Pubmed the manuscript submissions to our journal trippled. I would like to thank to all the scientists who gives us the oppurtunity to evaluate their work. The visibility and fulltext download of our articles exceeded 1500 during the first half of September 2017. This year our journal received second highest number of submissions in its history. In 2013 we have received 228 manuscripts and in 2017 we have received 200 manuscripts. The manuscripts submitted are mostly in the field of Perinatology followed by Reproductive Endocrinology and infertility, Gynecological Oncology and Urogynecology.

Our acceptance rate was 26% in 2017. We try to be constructive and provide feedback to the authors about possible sources of bias, methodological flaws, reporting errors and we encourage the authors to write the limitations of their studies. I think this provides an area of scientific discussion among colleagues and improve experience of every one of us even the manuscripts are not accepted. We would like to thank to our 228 reviewers and our Editorial board for donating their time to our journal just for improvement of science. In 2018 we are planning a series of Systematic Review and Metaanalysis courses for encouraging scientists to evaluate the literatüre and provide a highest level of scientific information for the readers of our journal. Please save the date of first course on 17-18 February 2018. Those who wants to join this team can mail to me.

I wish all the best in the new year.

Eray Çalışkan
Editor in Chief

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2017;14:199-202

Comparison of corifollitropin alfa and daily recombinant follicle-stimulating hormone in poor responder patients undergoing *in vitro* fertilization cycles

Düşük over yanıtlı hasta grubunda corifollitropin alfa ve rekombinant folikül uyarıcı hormonun in vitro fertilizasyon sikluslarında karşılaştırılması

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Abstract

Objective: The aim of this study was to compare the effect of corifollitropin alfa (CFA) and recombinant follicle-stimulating hormone (rFSH) in poor-responder patients undergoing antagonist cycles.

Materials and Methods: The study was a retrospective analysis of the treatment results of 214 poor responder patients who had been admitted to the *In Vitro* Fertilization Unit of İzmir Medical Park Hospital between November 2014 and November 2016. Intracytoplasmic sperm injections were performed in 38 patients (group 1) with CFA, and the remaining 176 (group 2) with rFSH for controlled ovarian hyperstimulation.

Results: The age, body mass index, anti-müllerian hormone level, duration of infertility, duration of induction and antral follicle number were similar in the two groups. There was no difference in the total aspirated oocyte counts, mature oocyte ratio, fertilization rate, implantation rate, and clinical pregnancy rates between the two groups. The implantation rate was 9/38 (23.6%) in group 1 and 42/176 (23.8%) in group 2, whereas the clinical pregnancy rates were 16.3% and 17.2%, respectively.

Conclusion: No difference was found in terms of oocyte count, fertilization rate, implantation rate, and clinical pregnancy rates of CFA or rFSH use in the antagonist cycles in poor-responder patients.

Keywords: Corifollitropin alfa, diminished ovarian reserve, gonadotropin-releasing hormone antagonist, *in vitro* fertilization, poor responder

Öz

Amaç: Bu çalışmanın amacı düşük over yanıtlı hasta grubunda antagonist sikluslarda korifollitropin alfa (CFA) ve rekombinant folikül stimülant hormonun (rFSH) etkinliğini karşılaştırmaktı.

Gereç ve Yöntemler: Çalışma İzmir Medical Park Hastanesi *İn Vitro* Fertilizasyonu Merkezi'nde Kasım 2014 ile Kasım 2016 tarihleri arasında düşük over yanıtlı toplam 214 hastanın kayıtlarından retrospektif olarak yapıldı. Otuz sekiz hastaya CFA (grup 1), 176 hastaya (grup 2) rFSH kulanılarak kontrollü ovaryan stimülasyon yapıldı.

Bulgular: Yaş, vücut kitle indeksi, anti-müllerian hormon düzeyi, infertilite süresi, indüksiyon süresi ve antral follikül sayısı her iki grupta aynıydı. Aspire edilen toplam oosit sayısı, matür oosit oranı, fertilizasyon oranı, implantasyon oranı ve klinik gebelik oranı açısından her iki grup arasında fark gözlenmedi. İmplantasyon oranı grup 1'de 9/38 (%23,6) ve grup 2'de 42/176 (%23,8) iken; klinik gebelik oranı sırasıyle grup 1'de %16,3 ve grup 2'de %17,2 idi.

Sonuç: Düşük over yanıtlı hasta gruplarında yapılan antagonist sikluslarda CFA ve rFSH kullanımının oosit sayısı, fertilizasyon oranı, implantasyon oranı ve klinik gebelik oranı arasında fark yoktur.

Anahtar Kelimeler: Korifollitropin alfa, yumurtalık rezervinin azalması, gonadotropin salgılatıcı hormon antagonisti, *in vitro* fertilizasyonu, düşük over yanıtlı

PRECIS: No difference was found in terms of oocyte count, fertilization rate, implantation rate, and clinical pregnancy rates of CFA or rFSH use in the antagonist cycles in poor-responder patients.

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Introduction

Corifollitropin alfa (CFA) is a new gonadotropin analogue with follicle-stimulating hormone (FSH) activity, which is effective for 7 days at the beginning and continuation of multi-follicular development[1]. This FSH analogue is a recombinant molecule that contains the carboxy terminal peptide structure of the human FSH beta subunit, but does not exhibit luteinizing hormone (LH) activity that affects only FSH receptors[1,2]. The greatest advantage of this molecule is the half-life of about 68 hours[3]. The pharmacodynamic properties of this molecule are that the serum concentration reaches the peak level ($T\text{-}_{max}$) in a short time and reaches the maximum concentration ($C\text{-}_{max}$) after 25-45 hours of injection[4]. There are no adverse effects or complications associated with this drug, which is well tolerated by patients[5]. The treatment of patients with low over-exposure has been the main topic of many randomized studies in past years. Different treatment regimens have been applied to increase over-response and pregnancy rates. Decreasing oocyte quality and decreasing over-reserve are closely related to female age[6-8]. The European Society of Human Reproduction and Embryology developed a new definition called Bologna criteria in 2011 for the poor-response patient group[9]). These criteria are: 1) older age of women (40 years) or other existing factors causing diminished ovarian reserve (DOR); 2) less oocyte counts in treatments taken in previous treatments (3 oocytes); 3) disorder in over reserve tests [antral follicles <5-7 or anti-müllerian hormone (AMH) <0.5-1.1 ng/mL]. Two of these criteria will cause the patient to have a diagnosis of DOR[9].

Materials and Methods

This study was the result of a retrospective study of 214 patients who were diagnosed as having DOR according to the Bologna criteria at the *in vitro* fertilization (IVF) Unit of İzmir Medical Park Hospital between November 2014 and November 2016. Ethics committee approval was obtained from İzmir University Ethics Committee (approval number: 012742) before the study commencement. Informed consent was obtained from all of the study participants. The inclusion criteria were: being aged <45 years old, regular menstrual cycles (24-35 days), body mass index of 18-30 kg/m^2, absence of any endocrine pathology, no severe male factor (total progressive motile sperm count 1 million/mL (Table 1). Thirty-eight patients were treated with single-dose subcutaneous injection of 150 µg CFA (Elonva, NV Organon, Oss, Netherlands) on the second or third day of the cycle as the first day of controlled ovarian hyperstimulation (COH). On the seventh day of treatment, 300 IU of highly purified human menopausal gonadotrophin (Merional, IBSA, Switzerland or Menopur, Ferring, Turkey) were administered, similar to the protocol of Polyzos and Devroey[6], subcutaneously per day to each patient (group 1). The remaining 176 patients were treated with 300 IU follitropin alpha (Gonal-f; Merck, Switzerland) or follitropin beta (Puregon; NV Organon, Oss, Netherlands) subcutaneously on the second or third day of the cycle (group 2). In both groups, 0.25 mg gonadotropin-releasing hormone (GnRH) ganirelix (Orgalutran; NV Organon, Oss, Netherlands) or cetrorelix (Cetrotide; Merck, Switzerland) was given daily until the day of human chorionic gonadotropin (hCG) to prevent premature luteinization when the follicle diameter was 13 mm or more. When the leading follicle was 18 mm, 250 µg recombinant hCG (Ovitrelle; Merck, Switzerland) was administered subcutaneously for the final maturation of the oocyte. Thirty-five hours later, under general anesthesia, transvaginal ultrasound-guided oocyte pick-up was performed. Intracytoplasmic sperm injections was applied to all patients. All embryos were cultured for three days *in vitro* and then transferred in the presence of transabdominal ultrasonographic guidance. Four hundred milligrams per day of micronized progesterone (Progestin capsules 200 mg, Koçak Farma, İstanbul, Turkey) and 90 mg progesterone gel (Crinone gel, Merck, Switzerland) were applied vaginally for luteal phase support. On the second day of the cycle, serum estradiol, LH, and FSH, and on the day of hCG treatment, estradiol, LH, and progesterone values were recorded. On the second, seventh, and hCG days of the cycle, follicle evaluation was performed using transvaginal ultrasonography. Twelve days after the embryo transfer, a beta-hCG test was performed. Transvaginal ultrasonographic evaluation was performed for fetal heart beat two weeks after a finding a positive hCG test.

Statistical Analysis

The Statistical Package for the Social Sciences program (SPSS 20) [International Business Machines (IBM) Corp. released 2011. IBM SPSS Statistics for Windows, version 20.0, Armonk, NY: IBM Corp.] was used to evaluate the data. Variables mean + standard deviation and median (minimum-maximum) percentage and frequency values were used. The homogeneity of the variances from the preconditions of the parametric tests was checked using the Levene test. The assumption of normality was examined using the Shapiro-Wilk test. The differences between the two groups were evaluated using Student's t-test

Table 1. Demographic characteristics of patients

	Grup 1	Grup 2	p
Patient number	25	119	
Age (year)	39.2	38.1	0.954
BMI (kg/m^2)	26.1	25.4	0.687
Duration of infertility (year)	5.3	5.7	0.545
Duration of induction (day)	10.5	10.3	0.998
Number of antral follicule	5.1	5.2	0.997
Endometrial thickness (hCG day)	8.1	8.3	0.995
AMH (ng/dL)	0.81	0.69	0.412
Progesteron (hCG day µg/L)	1.32	1.45	0.124

BMI: Body mass index, AMH: Anti-müllerian hormone, hcG: Human chorionic gonadotropin

when parametric test prerequisites were provided, and the Mann-Whitney U test was used when the conditions were not met. Categorical data were analyzed using the maximum likelihood and chi-square tests. In cases where the expected frequencies were less than 20%, the Monte Carlo Simulation Method was used including these frequencies in the analysis. For the significance level of the tests, p<0.01 was accepted.

Results

CFA (group 1) was applied to 38 of 214 patients with DOR, as diagnosed according to the Bologna criteria, and recombinant FSH and antagonist protocol (group 2) treatment was applied to the remaining 176 patients. It was not possible to retrieve any oocytes in 13 (34.2%) patients in group 1 and 57 (32%) patients in group 2; therefore, these cycle were cancelled (p=0.718). The mean number of oocytes collected in group 1 with 25 patients with at least one oocyte was 3.2, and in group 2 with 119 patients it was 3.4 (p=0.879). The mean number of mature oocytes metaphase 2 was 1.8 in group 1 and 1.6 in group 2 (p=0.745). The duration of stimulation was 10.52 days in group 1 and 10.34 days in group 2 (p=0.894). The mean number of transferred embryos was 1.6 in group 1, whereas this value was 1.5 in group 2 (p=0.478). The implantation rate was 9/38 (23.6%) in group 1 and 42/176 (23.8%) in group 2 (p=0.578), and the clinical pregnancy rate was 16.3% and 17.2%, respectively (p=0.622). Multiple pregnancy and drug adverse effects were not observed in any patients (Table 2).

Discussion

Since 2008, there have been different results in a limited number of studies on single-dose CFA administration. In 2008, Devroey et al.[10] wrote the first study about CFA. The authors suggested that a single injection of corifollitropin alfa induced a dose-related increase in multifollicular development and in the number of retrieved oocytes[11]. Devroey et al.[10] postulated that CFA was a novel and effective treatment option for potential normal-responder patients undergoing ovarian stimulation with GnRH antagonist co-treatment for IVF resulting in ongoing pregnancy rates equal to that achieved with daily rFSH. Mahmoud Youssef et al.[12] published a meta-analysis in 2012. They included four randomized trials involving 2326 women. There was no evidence of a statistically significant difference in ongoing pregnancy rates for CFA versus rFSH[12]. Boostanfar et al.[13] designed a large comparative randomized double-blind trial that confirmed the non-inferiority of pregnancy rates for CFA compared with recombinant FSH in a GnRH antagonist COH protocol in advance-age patients undergoing IVF. CFA was proven noninferior to daily rFSH with respect to cardiopulmonary resuscitations, number of oocytes retrieved, and live birth rates, and the drug was generally well tolerated[13]. Another study was designed by Polyzos et al.[14] with poor ovarian responders. In this study, the Bologna criteria were used to enroll the patients. The protocol with CFA in this group of patients resulted in low poor responder (PR) similar to the conventional short agonist protocol[14]. Revelli et al.[15] demonstrated that starting CFA on day 4 of the cycle resulted in comparable PR with significantly less injections and a similar risk of Ovarian Hyperstimulation syndrome (OHSS)[15]. On the other hand Oehninger reported that, in women aged 35 to 42 years, the prediction of ovarian response to CFA treatment was related with AMH, AFC, and age at the start of stimulation for in both high and PR patients[16]. In addition, basal FSH values for high ovarian response and menstrual cycle length for poor ovarian response were prognostic. In a meta-analysis consisting of 2138 women who were randomized to receive corifollitropin alfa and 1788 who were randomized to receive daily rFSH, Fensore et al.[17] emphasized that the risk of cycle cancellation due to overstimulation was significantly higher in the CFA group. On the other hand, the incidence of OHSS was comparable between patients receiving long-lasting or daily rFSH. Accordingly, they suggested that CFA resulted in a higher number of metaphase 2 oocytes collected and a higher number of cycles cancelled due to overstimulation; therefore, CFA should be cautiously considered in women with the potential of being hyper-responders. In view of this meta-analysis, one can consider using CFA more effectively for patients with DOR, but to date, the results have not been consistent. In a retrospective study, Polyzos et al.[14] used HP-hMG as an additional gonadotropin in poor-responder patients according to the Bologna criteria. They achieved a very reasonable ongoing pregnancy rate (28%) in patients aged below 40 years, whereas no pregnancies occurred in patients aged over 40 years.

Table 2. Over response, embryo results and pregnancy rates

	Grup 1	Grup 2	p
Patient Number	38	176	
Number of cycles cancelled	13 (34.2%)	57 (32%)	0.72
Number of taken oocytes	3.2	3.4	0.88
MII Number	1.8	1.6	0.75
2PN Number	1.5	1.4	0.99
Emb Grade			
Grade 1-2	11 (44%)	48 (40.3%)	0.74
Grade 3-4	14 (56%)	71 (59.7%)	0.74
Transferred embriyo number	1.6	1.5	0.48
Pozitive hCG	9 (23.6%)	42 (23%)	0.58
Clinical pregnancy	16.3%	17.2%	0.64

Emb: Endometrial biopsy, 2PN: Two-pro-nucleii, hcG: Human chorionic gonadotropin MII: Metaphase II

Study Limitations

The retrospective design is the major limitation of our study. Therefore, prospective randomized studies are also necessary about this subject.

Conclusion

CFA is a long-acting novel gonadotropin for COH. In our study, we used a similar protocol to Polyzos et al.[14] who found considerably high PR in patients with DOR, particularly in the younger age group. Unfortunately, we could not show any difference between the two different COH modalities. Although the CFA treatment was well accepted by the patients, the high cost of the medication was a limiting concern. As far as we know, this is the first study to report the use of CFA in patients with DOR from our country.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from İzmir University Ethics Committee (approval number: 012742)

Informed Consent: This study was the result of a retrospective study of 214 patients who were diagnosed as having DOR according to the Bologna criteria at the *In Vitro* Fertilization Unit of İzmir Medical Park Hospital between November 2014 and November 2016.

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Authorship Contributions

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

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Role of positron emission tomography-computed tomography in endometrial cancer

Endometriyal kanserde pozitron emisyon tomografisi-bilgisayarlı tomografinin rolü

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Abstract

Objective: The efficacy of preoperative ^{18}F-fluoro-D-glucose (^{18}F-FDG) positron emission tomography-computed tomography (PET-CT) in endometrium cancer is controversial. We examined the efficacy of PET-CT and the association between maximum standardized uptake value (SUV_{max}) and prognostic factors in endometrial cancer.

Materials and Methods: Thirty patients with endometrial cancer underwent preoperative ^{18}F-FDG/PET-CT. The patients were treated with abdominal hysterectomy with bilateral salpingo-oophorectomy, and bilateral systemic pelvic lymphadenectomy was planned for all patients; paraaortic lymphadenectomy was performed in patients with intermediate and high risk. Tumor histology, grade, depth of myometrial invasion, maximum tumor diameter, lymphovascular invasion, nodal status, and ovarian/adnexal metastases were recorded.

Results: The mean primary tumor diameter was reported smaller in PET-CT and the effect size of PET-CT was -0.60. The kappa value was 0.06 for myometrial invasion. Pelvic lymph node metastasis was reported in 22.2% of patients in PET-CT. However, 3.7% of patients had pelvic lymph node metastasis. The kappa value for pelvic lymph node metastasis was 0.23, and sensitivity, specificity, and positive and negative predictive values were 100%, 80.7%, 16.6%, and 100%, respectively. Paraaortic lymph node metastasis in PET-CT was suspected in 10%. However, paraaortic lymph node metastasis was found in 6.7% in histopathologic analyses. The kappa value was 0.15. The sensitivity, specificity, and positive and negative predictive values of PET-CT for detecting paraaortic lymph node metastases were 100%, 93.7%, 66.6%, and 100%, respectively. Myometrial invasion and tumor diameter were the only important prognostic factors affecting SUV_{max}.

Conclusion: According to our results, PET-CT has a limited role and diagnostic efficacy in endometrial cancer. The indications of FDG/PET-CT in endometrium cancer should be studied further and revised.

Keywords: Endometrial cancer, maximum standardized uptake value, positron emission tomography, diagnosis, prognostic factors

Öz

Amaç: Endometriyal kanserde preoperatif ^{18}F-floro-D-glukoz (^{18}F-FDG) pozitron emisyon tomografi-bilgisayarlı tomografinin (PET-BT) etkinliği tartışmalıdır. Endometriyal kanserde PET-BT'nin etkinliğini ve maksimum standart uptake değeri (SUV_{maks}) ile prognostik faktörler arasındaki ilişkiyi inceledik.

Gereç ve Yöntemler: Endometriyal kanserli otuz hastada preoperatif ^{18}F-FDG/PET-BT vardı. Hastalar bilateral salpingo-oofrektomi ile abdominal histerektomi ile tedavi edildi ve her olguda bilateral sistemik pelvik lenfadenektomi planlandı, orta ve yüksek riskli hastalarda paraaortik lenfadenektomi yapıldı. Tümör histolojisi, grade, myometrial invazyon derinliği, maksimum tümör çapı, lenfovasküler invazyon, nodal durum, over/adneksiyel metastaz kaydedildi.

Bulgular: Ortalama primer tümör çapının PET/BT'de daha küçük olduğu ve PET-BT'nin etki büyüklüğünün 0,60 olduğu rapor edildi. Myometrial invazyon için kappa değeri 0,06 idi. Pelvik lenf nodu metastazı PET-BT'de %22,2 olarak bulundu. Bununla birlikte, hastaların %3,7'sinde pelvik lenf nodu metastazı vardı. Pelvik lenf nodu metastazı için kappa değeri 0,23, duyarlılık, özgüllük, pozitif prediktif değeri ve negatif prediktif değeri sırasıyla %100, %80,7, %16,6 ve %100 idi. PET-BT'de, paraaortik lenf nodu metastazı açısından %10'unda şüphelenildi. Bununla birlikte, histopatolojik analizlerde paraaortik lenf nodu metastazı %6,7'sinde bulundu. Kappa değeri 0,15 idi. PET-BT'nin paraaortik lenf nodu metastazlarını saptamaya yönelik sensitivitesi, spesifitesi, pozitif prediktif değeri ve negatif prediktif değeri sırasıyla %100, %93,7, %66,6 ve %100 idi. SUV_{maks}'ı etkileyen tek önemli prognostik faktör, myometrial invazyon ve tümör çapı idi.

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Öz

Sonuç: Sonuçlarımıza göre, PET-BT'nin endometriyum kanserinde sınırlı bir rolü ve tanısal etkinliği vardır. FDG/PET-BT'nin endometriyum kanseri endikasyonları daha fazla araştırılmalı ve gözden geçirilmelidir.
Anahtar Kelimeler: Endometriyal kanser, maksimum standart uptake değeri, pozitron emisyon tomografi, tanı, prognostik faktörler

PRECIS: Positron emission tomography-computed tomography has a limited role and diagnostic efficacy in endometrial cancer.

Introduction

Endometrial cancer is the most common gynecologic cancer; the estimated new cases and deaths in 2015 were 54.870 and 10.170, respectively[1]. The majority of cases are confined to the uterus and diagnosed at an early stage[1]. Endometrium cancer spreads primarily by direct extension, lymphatic channels, trans tubal migration, and blood vessels. The main lymphatic metastases involve pelvic lymph nodes, and less commonly presacral, paraaortic, and inguinal lymph nodes[2]. Until 1988, endometrium cancer was staged clinically[3]. After finding evidence that clinical staging was inaccurate, the International Federation of Gynaecology and Obstetrics (FIGO) staging of endometrium cancer changed to a surgical-pathologic-based system. Surgical staging of endometrium cancer provides prognostic factors that are not available before surgery, or which are not always in exact concordance before and after surgery. Prognostic factors are the stage, tumor histology, grade, depth of myometrial invasion, lymphovascular space invasion (LVSI), tumor diameter, extrauterine spread, and lymph node metastasis[2,3].

Positron emission tomography (PET) uses the detection of enhanced glucose metabolism in malignant tumors based on the uptake of ^{18}F-fluoro-D-glucose (^{18}F-FDG) for functional diagnosis of malignant tumors. PET-computed tomography (CT) allows simultaneous imaging of anatomic and metabolic information. ^{18}F-FDG/PET-CT has been widely used in clinical practice for tumor detection, staging, treatment monitoring and detection of disease recurrence. Preoperative PET-CT imaging is not universally accepted in endometrial cancer, and the efficacy and accuracy of ^{18}F-FDG/PET-CT in endometrium cancer is controversial and may be overestimated. In this study, we examined the efficacy of PET-CT in a non-stratified patient group with endometrium cancer in a tertiary university setting over a 1-year period. We also analyzed the association between maximum standardized uptake values (SUV_{max}) and prognostic factors based on final pathology.

Materials and Methods

Subjects

Patients diagnosed as having endometrial cancer histopathologically who underwent PET-CT for treatment planning in our department between 2014 and 2015 were included in the study. Patients who did not undergo surgery or preoperative PET-CT imaging were not recruited. Patients who had neoadjuvant treatment before the operation, claustrophobia, and uncontrolled diabetes mellitus (random blood sugar >200 mg/dL) were excluded. The standard preoperative procedures included clinical examination, chest X-ray, and abdominal and vaginal ultrasonography. Laboratory examinations including routine hematology, biochemistry, cancer antigen-125 (CA-125) were performed. Routine preoperative imaging for myometrial invasion was not performed because it was not found to be cost-effective. The study was approved by Süleyman Demirel University Ethics Committee (approval number: 54).

Surgery

All patients had a midline laparotomy and abdominal hysterectomy with bilateral salpingo-oophorectomy under general anesthesia. Panniculectomy was performed in two patients for staging purposes. Bilateral systemic pelvic lymphadenectomy was planned for all patients; paraaortic lymphadenectomy was performed in patients with intermediate and high risk. Lymph node dissection was performed en-bloc without dividing for separate regions for oncologic safety. Suspicious nodes on FDG/PET-CT were marked. Analysis of matching for the PET-CT and histopathology was made for the whole nodal chain including the marked lymph node(s).

Positron emission tomography-computed tomography protocol and image analysis

Whole-body ^{18}F-FDG/PET-CT images were performed using a PET/CT scanner (Philips Gemini TF), consisting of dedicated lutetium yttrium oxyorthosilicate full-ring PET scanner and 64-slice CT. All patients were kept fasting for six hours before intravenous (i.v.) injection of 3.7 MBq/kg (0.1 mCi/kg) of ^{18}F-FDG. During the 60 min. waiting period, all patients were orally hydrated with around 1.5 L of contrast. After 60 min., the combined examination was started by asking the patients to empty their bladder and injecting the i.v. contrast. The CT scan was acquired first, followed by the PET scan. PET and CT images (non-corrected and attenuation-corrected) were evaluated in the rotating maximum-intensity projection and in the cross-sectional planes view (transverse-sagittal-coronal). The ^{18}F-FDG uptake in the primary tumor was quantified using SUV_{max} measurements.

Pathological evaluation

Tumor histology, grade, depth of myometrial invasion, maximum tumor diameter, lymphovascular invasion, nodal status, ovarian/adnexal metastases, and cytology were recorded. Each primary tumor and dissected lymph nodes were sliced and stained with hematoxylin and eosin (H&E) and examined

microscopically by at least one gynecologic pathologist. Pathologist(s) were blinded to the PET/CT results. Discordant results on lymph nodes were further evaluated by ultra-staging. Ultra-staging involved cutting an additional two adjacent 5-µm sections at 2 levels 50-µm apart from each paraffin block that lacked metastatic carcinoma on routine H&E staining. At each level, one slide was stained with H&E and with immunohistochemistry using anti-cytokeratin.

Prognostic factors

Prognostic factors evaluated were tumor histology, grade, depth of myometrial invasion, maximum tumor diameter, lymphovascular invasion, nodal status, elevated CA-125, and thrombocytosis. Grade 1 and 2 tumors were classified as low grade; grade 3 tumors were classified as high grade.

Statistical Analysis

Statistical analyses were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium) and p values less than 0.05 were considered statistically significant.

Analyses of consistency and agreement between positron emission tomography-computed tomography and histopathology: Continuous variables

The mean and standard derivation of continuous variables were calculated using the t-test. The importance of the difference between means was evaluated through effect size. Effect size measures how much the mean in PET-CT exceeded the mean of the histopathologic measurement. Effect size <0.20 was considered small; 0.50 was moderate effect size, and >0.80 was large effect size. The consistency and agreement of the continuous variables (tumor diameter) were evaluated using the intraclass coefficient and concordance correlation coefficient. Concordance correlation coefficient <90 indicates poor correlation and agreement. Intraclass correlation coefficient (ICC) was analyzed for absolute agreement and consistency. ICC were calculated for each single measurement and also for average (mean) values; ICC >70 indicated poor agreement, >80 indicated moderate agreement, >90 good agreement, and 100 meant perfect agreement.

Analyses of consistency and agreement between positron emission tomography-computed tomography and histopathology: Ordinal and nominal variables

The frequency of variables was calculated, and kappa statistics was used to analyze agreement between PET-CT and histopathologic findings. Kappa value is 1 when there is perfect agreement between PET-CT findings and histopathologic findings. Kappa value is zero when there is no agreement better than chance. Kappa value is negative when the agreement is worse than chance. Kappa index (inter-rater agreement concordance, in ordinal or nominal scales) is usually interpreted according to qualifiers as "poor" (<0.20), "slight" (0.20-0.40), "fair" (0.41-0.60), "moderate" (0.61-0.80), and 0.81-1 as almost perfect agreement.

Analyzes of maximum standardized uptake value and high-risk prognostic indicators

Median and interquartile ranges SUV_{max} for each prognostic indicator such as histology, grade, myometrial invasion, lymphovascular invasion, and lymph node metastasis were calculated. The Mann-Whitney U test or Kruskal-Wallis test was used to analyze primary tumor SUV_{max} and prognostic indicators. Regression analyses were used to study the relation between tumor diameter in pathologic measurement and primary tumor SUV_{max}.

Results

A total of 30 patients were included in the study. The mean age of patients was 58.8±9.3 years. Eighty-six percent of the women were postmenopausal. Endometroid histology was reported in 90% of patients. Serous histology was reported in 10% of patients. Tumor was grade 1, 2, and 3 in 43.3%, 40%, and 16.7%, respectively. LVSI was observed in 6.7% of cases. Pelvic lymphadenectomy was planned for all patients. However, the procedure was abandoned during the operation in two patients because of co-morbidities and was not technically feasible and safe in one patient. Pelvic and paraaortic lymphadenectomy were performed in 27/30 and 18/30 of patients, respectively. The mean number of pelvic lymph nodes harvested was 18.6±10.6. The mean number of harvested paraaortic lymph nodes was 10.5±5.4. The mean blood glucose level was 110±23.1 mg/dL. The mean CA-125 level was 25.5±39.1 IU/mL. Thirteen percent of patients had CA-125 higher than the reference value of 35 IU/mL. Seven percent of patients had thrombocytosis. There was no positive cytology.

Agreement between positron emission tomography-computed tomography and pathologic findings

The mean primary tumor diameter was reported as 4.2±1.8 cm in PET-CT. The man primary tumor diameter was measured as 5.8±3.2 cm in the specimen. The effect size was found to be -0.60, indicating a modest change in diameter of tumor in PET-CT and histopathology. Tumor diameter was poorly correlated with measurements taken in PET-CT. Consistency and absolute agreement of PET-CT and histopathologic analyses are shown in Table 1 and Figure 1.

Thirty-three percent had myometrial invasion in PET-CT; however, 93.3% had myometrial invasion in the specimen. A kappa value of 0.06 [95% confidence interval (CI): -0.0303-0.168] was found for myometrial invasion in PET-CT and histopathology.

Pelvic lymph node metastasis was reported in 22.2% in the PET-CT reports. However, 3.7% of patients had pelvic lymph node metastasis. The kappa value for pelvic lymph node metastasis was 0.23 (95% CI: -0.15-0.62). There was fair consistency between PET-CT and histopathologic findings, and the sensitivity, specificity, and positive and negative predictive values were 100%, 80.7%, 16.6%, and 100%, respectively. Paraaortic lymph node metastasis in PET-CT was suspected in

10%. However, paraaortic lymph node metastasis was found in 6.7% in histopathologic analyses. The kappa value was 0.15 (95% CI: -0.0749-0.387). There was no agreement between PET-CT and histopathologic findings. The sensitivity, specificity, and positive and negative predictive values of PET-CT for detecting paraaortic lymph node metastases were 100%, 93.7%, 66.6%, and 100%, respectively.

Factors effecting primary tumor maximum standardized uptake value

Myometrial invasion and tumor diameter were the only significant factors affecting SUV_{max} (Tables 2, 3). The median SUV_{max} of tumors with no myometrial invasion was 5.2. However, the median SUV_{max} of tumors with myometrial invasion less than 50% and more than 50% were 10.6 and 18.4, respectively (Figure 2). Primary SUV_{max} was not statistically significantly higher in high grade, LVSI positive, and lymph node positive patients. Tumor size and SUV_{max} were correlated; as the tumor size enlarged, SUV_{max} values increased (Table 3). SUV_{max} was not affected by high CA-125 values or thrombocytosis.

Discussion

Pre-operative imaging in endometrium cancer may help to plan surgery by adding or omitting staging procedures. Secondly, imaging may help to identify candidates for fertility-sparing treatment. Another indication of pre-operative imaging could be in patients who cannot undergo surgery because of co-morbidities[4]. We found the efficacy of ^{18}F-FDG/PET-CT was low in endometrium cancer, and ^{18}F-FDG/PET-CT was not in concordance with histopathologic findings. We also analyzed prognostic factors that affected SUV_{max} values. The number of studies in this regard is few and the results conflicting[5,6]; most detected only lymph node metastasis, rather than other clinicopathologic prognostic factors.

Women with endometrium cancer are stratified into risk groups for recurrence and treatment planning. The French Multicenter Collaboration study and others identified that tumor size larger than 2-3 cm was a poor prognostic factor[7-9]. Tumor size is correlated with myometrial invasion, nodal metastases, peritoneal cytology, CA-125 levels, advanced disease, and relapse[8,9]. The size of the primary tumor was moderately consistent with histopathologic tumor measurement, and tumor diameter was predicted smaller in PET-CT scans in our study.

Myometrial invasion is a part of staging in endometrium cancer, and PET-CT was not useful in predicting myometrial invasion; 33% of patients had myometrial invasion in PET-CT, whereas 93.3% had myometrial invasion in the specimen. The sensitivity and specificity of CT in detecting myometrial invasion range from 40% to 83% and 42% to 75%, respectively[10]. Sudo et al.[11] reported that patients with endometrium cancer should first be triaged by myometrial invasion in magnetic resonance imaging (MRI) before PET-CT. Their report on 37 patients with endometrium cancer showed that PET-CT could identify risk groups after MRI triage. FDG/PET-CT has limitations in the evaluation of the depth of myometrial invasion and in defining tumor borders[12].

Lymphatic metastases are one of the most important prognostic factors in endometrium cancer. There are various imaging modalities to assess for lymph node metastasis before surgery. Park et al.[13] compared PET-CT and MRI to detect lymph node metastases in patients with endometrial cancer, and reported that the sensitivity and specificity of FDG/PET-CT was better than MRI for detecting metastatic lymph nodes in patients with endometrial cancer. Pelvic and paraaortic lymph node metastases were over-estimated by FDG/PET-CT in our study. We found fair consistency for pelvic lymph nodes and no consistency for paraaortic lymph nodes. Our findings for

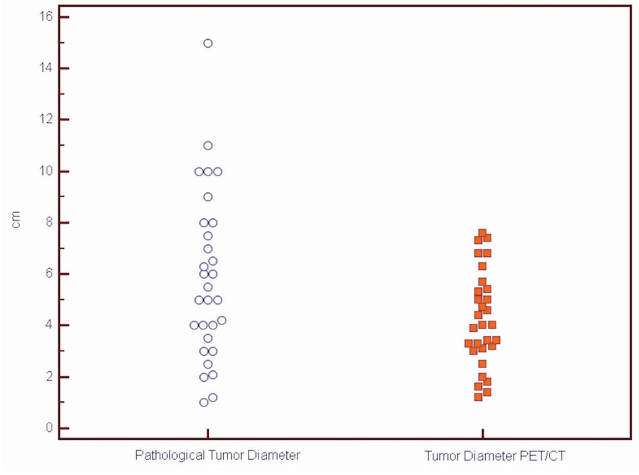

Figure 1. Tumor size estimate in positron emission tomography-computed tomography and histopathologic measurement

PET/CT: Positron emission tomography/Computed tomography

Table 1. Intraclass correlation coefficient analyses for tumor diameter

Consistency	Intraclass correlation[a]	95% Confidence interval
Single measures[b]	0.64	0.37 to 0.81
Average measures[c]	0.78	0.54 to 0.89
Absolute	**Intraclass correlation[a]**	**95% Confidence interval**
Single measures[b]	0.51	0.19 to 0.73
Average measures[c]	0.67	0.32 to 0.84

[a]The degree of absolute agreement among measurements, [b]Estimates the reliability of single ratings, [c]Estimates the reliability of averages of κ ratings

detecting lymph node metastases using FDG/PET-CT are in agreement with other studies. Gholkar et al.[14] reported that FDG/PET-CT had a sensitivity of 100%, specificity of 61.11%, positive predictive value of 22.22%, negative predictive value of 100%, and accuracy of 65% for pelvic lymph node metastases; sensitivity of 100%, specificity of 66.67%, positive predictive value of 20%, negative predictive value of 100%, and accuracy of 69.23%[15].The high sensitivity and negative predictive value of FDG/PET-CT are important in the detection of patients who are truly lymph node-negative[15]. However, FDG/PET-CT has a high false positive rate for lymph nodes. Besides, FDG/PET-CT cannot detect metastatic lymph nodes smaller than 5 mm[15].

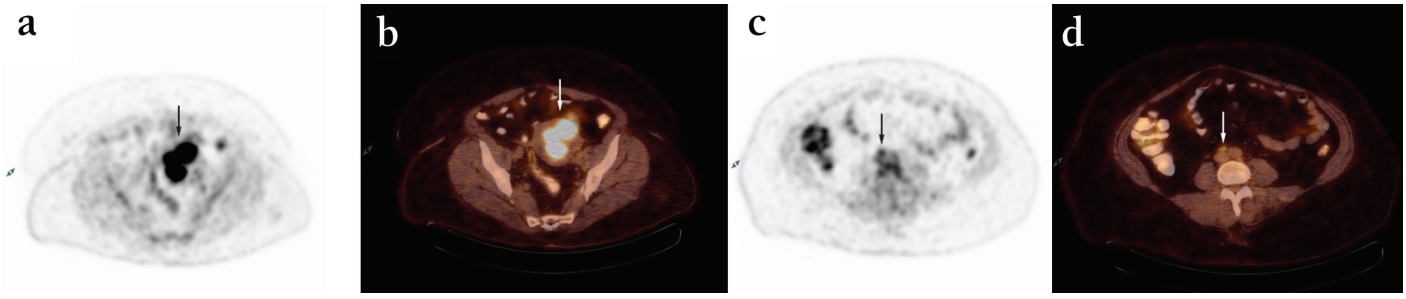

Figure 2. A 60-year-old female with endometrial cancer. Fluoro-D-glucose/positron emission tomography-computed tomography images demonstrate increased fluoro-D-glucose uptake in the tumor (a,b) and metastatic inter aortocaval lymph node (c,d)

Table 2. Maximum standardized uptake value and histopathologic prognostic factors

		SUV_{max} median	SUV_{max} IQR	p
Histology	Endometroid	14.2	9.8	0.51
	Serous	15.8	7	
Grade	Low (grade1/2)	13.3	9.8	0.16
	High (grade 3)	15.8	7	
Myometrial invasion	No invasion	5.2	4.5	0.03
	Invasion	15.1	9.2	
LVSI	Negative	15.1	10	0.66
	Positive	11.7	3.7	
Myometrial invasion	(a) No	5.2	4.5	Different from c
	(b) <50%	10.6	8.2	Different from c
	(c) >50%	18.4	4	Different from a, b 0.004
Lymph node metastasis	Negative	15.1	9.5	NA
	Positive	16.2	-	
CA-125	Normal	13.6	10.1	0.37
	Elevated	15.5	8.4	
Thrombocyte	Normal	14.9	8.6	1
	Thrombocytosis	14.2	-	

p<0.05 statistically significant
NA: Non-applicable, statistical evaluation not appropriate, LVSI: Lymphovascular space invasion, CA-125: Cancer antigen-125, IQR: Interquartile range, SUV: Standardized uptake values

Table 3. Regression analyses between primary tumor maximum standardized uptake value and tumor diameter
primary tumor maximum standardized uptake value=8.03+1.06 diameter

Parameter	Coefficient	Standard error	95% CI	t	p
Intercept	8.03	2.10	3.69 to 12.38	3.81	0.0008
Slope	1.06	0.30	0.44 to 1.68	3.53	0.0017

CI: Confidence interval

Analyses of prognostic factors and SUV_{max} values showed that SUV_{max} was significantly higher in patients with deeper myometrial invasion and higher tumor diameter. However, we found no correlation between SUV_{max} and tumor histology, tumor grade, lymphovascular invasion, and lymph node metastases in contrast to other reports[16,17]. Kitajima et al.[18] reported that SUV_{max} was not a good index for preoperative stratifying patients into high risk and low risk, and suggested the use of metabolic tumor volume and total lesion glycolysis instead of SUV_{max}.

Study Limitations

The limitations of our study were similar to other reports; its design was retrospective, and a relatively small number of patients were included in a single tertiary center. Another limitation of our study may be that paraaortic lymphadenectomy was not performed in all cases due to technical considerations or due to other pathologic findings indicating low risk.

There are also diagnostic limitations of FDG/PET-CT in endometrial cancer due to the natural slow progress of the disease. The majority of patients with endometrial cancer have the endometrioid type, are early stage and low risk without lymph node metastases. The low prevalence of poor prognostic factors may cause a bias towards a lower positive predictive value and higher negative predictive value. We did not stratify patients by preoperative histopathologic findings or other imaging techniques. We rather preferred to include a non-stratified population of patients who were referred to the gynecologic oncology department in one year.

Conclusion

Surgical staging and obtaining prognostic factors using the FIGO staging is the standard of treatment for endometrial cancer, although lymphadenectomy for early-stage low-risk patients is controversial. Although the pre-operative use of PET-CT in endometrium cancer is increasing, the results of our study and others cast doubt on the diagnostic efficacy of PET-CT in endometrium cancer[19]. The indications of FDG/PET-CT in endometrium cancer should be revised and studied further.

Ethics

Ethics Committee Approval: This study was approved by Ethical Committee of Süleyman Demirel University (approval number: 54).

Informed Consent: Consent form was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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

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A comparison of normal and high post-void residual urine and urodynamic parameters in women with overactive bladder

Aşırı aktif mesanesi olan kadınlarda normal ve artmış işeme sonrası rezidülerin ve ürodinamik parametrelerin karşılaştırılması

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Abstract

Objective: To investigate voiding functions and assess the relationships of voiding parameters to overactive bladder symptoms and postvoiding residue volumes.

Materials and Methods: This is a retrospective study analyzing urodynamic parameters in patients who were diagnosed as having overactive in our urogynecology clinic between April 2014 and April 2016. A total of 290 women who met the selection criteria were included in the study. The patients were divided into two groups according to postvoiding residue volumes: group 1, postvoiding residue volumes <100 mL (n=135); group 2, postvoiding residue volumes ≥100 mL (n=155).

Results: A total of 290 women were included in the study; the mean age was 71.4 years. A total of 158 (54.5%) patients had detrusor over-activity during urodynamic testing. The mean maximum bladder capacity in elevated group 2 (postvoiding residue volumes ≥100 mL) was significantly higher than in group 1 (postvoiding residue volumes <100 mL) ($p<0.01$). Additionally, there was a significant difference between detrusor pressure at Q_{max} in both study groups ($p<0.05$). There were no significant differences in the first-sensation volume between the normal and elevated postvoiding residue volumes groups.

Conclusion: In conclusion, patients with overactive with elevated postvoiding residue volumes showed increased maximum bladder capacity, but detrusor over-activity was not more prevalent in these women compared with women with normal postvoiding residue volumes.

Keywords: Residue urine, urodynamics, voiding, bladder pressure

Öz

Amaç: Çalışmanın amacı işeme fonksiyonlarını ve işeme fonksiyonları ile aşırı aktif mesane semptomları ve işeme sonrası rezidü idrar volümü arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntemler: Çalışmada 2014 Nisan ile 2016 Nisan arasında ürojinekoloji kliniğimizde aşırı aktif mesane tanısı alan hastaların ürodinamik parametrelerini geriye dönük olarak değerlendirdik. Çalışmaya seçilme kriterlerini karşılayan 290 hasta dahil edildi. Hastalar işeme sonrası rezidü miktarlarına göre 2 gruba bölündü: Grup 1, rezidü <100 mL (n=135); grup 2, rezidü ≥100 mL (n=155).

Bulgular: Toplam 290 hasta çalışmaya dahil edildi ve hastaların ortalama yaşları 71,4 idi. Ürodinamik testler sonucunda toplam 158 hastada (54,5%) detrusor aşırı aktivitesi saptandı. Grup 2'deki (rezidü ≥100 mL) hastaların ortalama mesane kapasitesi grup 1'deki (rezidü <100 mL) hastalardan belirgin olarak yüksekti ($p<0,01$). Ayrıca 2 grup arasındaki maksimum akış hızındaki detrusor basınçları belirgin olarak farklıydı ($p<0,05$). Ancak 2 grup arasında hastaların ilk idrar hissi miktarında belirgin fark yoktu.

Sonuç: Sonuç olarak; artmış rezidü idrar volümüne sahip aşırı aktif mesaneli hastalarda artmış maksimum mesane kapasitesi gözlenirken, bu grupta işeme sonrası normal rezidüye sahip olan gruba göre detrusor aşırı aktivitesi prevelansı daha fazla değildi.

Anahtar Kelimeler: Rezidü idrar, ürodinami, işeme, mesane basıncı

PRECIS: We studied urodynamic parameters and their clinical importance in patients with overactive bladders.

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Introduction

Urinary incontinence is defined as involuntary leakage of urine[1,2]. It is estimated that between 26 and 61% of women receive care for urinary incontinence in their lifetime[3,4]. Overactive bladder (OAB) is a term that describes a syndrome of urinary urgency with or without incontinence, often accompanied by nocturia and urinary frequency[5,6].

Urodynamics comprises a group of tests used to evaluate urinary tract function. Urodynamic testing is a simple and non-invasive procedure for evaluating lower urinary tract symptoms. It is also a way of assessing maximum flow rate ($Q_{maximum}$; Q_{max}), average flow rate ($Q_{average}$; Q_{ave}), bladder capacity, and post-void residual (PVR) urine[7].

Voiding dysfunction is a broad term. It is defined by the International Urogynecological Assosication/International Continence Society as "incomplete micturition or abnormally slow micturition[8]." It is the cause of elevated PVR. Parameters for interpreting the results of PVR testing are neither standardized nor well evaluated. In general, a PVR of less than one-third of total voided volume is considered adequate emptying[9,10]. Additional parameters include designation of PVR greater than 100 mL as abnormal[11].

Urodynamic parameters and their clinical importance in patients with OAB have not been well studied. Therefore, we explored voiding functions and assessed the relationships of voiding parameters with OAB symptoms and PVR volumes.

Materials and Methods

The patients' data were retrospectively collected from the hospital medical records. We analyzed urodynamic parameters in patients who diagnosed as having OAB in our urogynecology clinic between April 2014 and April 2016. All patients signed informed consent forms. The same researchers (H.D. and C.H.) conducted retrospective chart reviews for all patients with OAB. A total of 290 women who met the selection criteria were included in the study. The patients were divided into two groups according to PVR: group 1, PVR <100 mL (n=135); group 2, PVR ≥100 mL (n=155).

OAB syndrome is defined as urinary urgency with or without urge incontinence, typically associated with increased daytime frequency and nocturia[12]. Detrusor over-activity (DO) is a urodynamic symptom characterized by involuntary detrusor contractions during the filling phase, which may be spontaneous or provoked[1].

Exclusion criteria were patients with pure stress incontinence, allergic diseases such as asthma, history of psychiatric disorders, urinary tract infection, urinary obstructive disease, metabolic diseases, neurologic disorders, and current use of diuretics.

All urodynamic tests were conducted by the same registered nurse and author (H.C.); the same instructions were used to prepare patients, and the same urodynamic machine was used for testing. Patients were evaluated using a multi-channel MMS® Solar (ADS, Ltd., Enschede, The Netherlands) urodynamic study device. Filling cystometry and uroflowmetry were performed. Also residual urine was measured. Isotonic saline was infused at a rate of 50 mL/min at room temperature for the filling. The patients were in the semi-sitting position. After the urinary bladder was full, volumes at first sensation of urination (mL), feeling the need to urinate (mL), feeling the need to urinate immediately (mL), bladder capacity (mL), and compliance (mL/cm H_2O) were measured. Detrusor contractions present after 15 cm H_2O that could not be inhibited were recorded. During the procedure, maneuvers such as coughing and straining were performed to assess whether urinary leakage from the external meatus occurred. The results of urodynamic tests were interpreted and reported by a urogynecologist (H.C.) using an identical reporting form. Catheters for vesical and abdominal pressures were reset before placement of the catheters.

The filling sensations assessed as volumes at first sensation; first urge sensation; strong urge sensation; and bladder capacity. After reaching cystometric bladder capacity, all women were given permission to void. Each woman was repositioned to an upright position. In this position they could void without abdominal pressure. After the measurement of pressures, PVR volume was measured by a nurse who attached a urethral catheter. Bladder capacity was calculated. The values of Q_{max} and detrusor pressure at Q_{max} were calculated from the pressure-flow testing.

Statistical Analysis

Statistical analysis was performed using the Number Cruncher Statistical System 2007 statistical software. The mean differences between groups were compared using Student's t-test, and the Mann-Whitney U and independent-samples t-test were applied for comparisons of median values. Logistic regression analyses were conducted to determine the association between voiding parameters and PVR volume. A p value less than 0.05 was considered statistically significant.

Results

A total of 290 women were included in the study; the mean age was 71.4 years. Table 1 shows the characteristics of the women. A total of 158 (54.5%) patients had DO during urodynamic testing. The mean maximum bladder capacity in elevated group 2 (PVR ≥100 mL) was significantly higher than in group 1 (PVR <100 mL) ($p<0.01$). Additionally, there was a significant difference between detrusor pressure at Q_{max} in both study groups ($p<0.05$). There were no significant differences in first-sensation volume between the normal and elevated PVR groups. Women with elevated PVR had significantly higher voided volume and maximum flow rates than did women with normal PVR. Additionally, we found no significant difference in DO between the two groups (p=0.282). Table 2 shows a comparison of urodynamic parameters through PVR volumes. Table 3 shows the results of logistic regression analysis to determine the best predictor(s) to discriminate between the groups.

Discussion

Urodynamic evaluation is a non-invasive process that provides objective and subjective data about bladder functioning. Measurements of urine flow and PVR with uroflowmetry are important parameters. There are some parameters that affect the urine flow rate including voided volume, detrusor contractibility, and bladder outlet obstruction. The purpose of this study was to evaluate urodynamic parameters in women who had OAB symptoms and either normal or elevated PVR volumes. There were 290 medical records for the analyses in the clinical setting.

During urodynamic testing, 158 (54.8%) women showed DO. In this study we find that nearly 50% of patients with OAB had DO on urodynamics testing. Our results are compatible with previous studies[11,13,14]. Additionally, Futyma et al.[15] observed DO in less than 20% of patients with OAB, which is much lower than the values in other studies where DO was found in more than 50% of patients. The authors believed that the difference in DO prevalence was associated with patients' performing maneuvers during the test that may have provoked urinary responses. The differences among studies may reflect differences in patient selection criteria before data analysis. Jeong et al.[16]

Table 1. Characteristics of the study groups

	Group 1 Normal PVR (<100 mL)		Group 2 Elevated PVR (≥100 mL)		p
	Mean ± SD/n-%	Median	Mean ± SD/n-%	Median	
Age	71.5±5.3	71	71.3±5.1	71	0.755
Vaginal parity (number)	2.5±1.3	2	2.4±1.5	2	0.271
Body mass index	31.0±5.0	31	30.4±4.8	30	0.302

PVR: Postvoiding residual urine, SD: Standart Deviation

Table 2. Comparison of urodynamic parameters through post-void residual volumes

	Group 1 Normal PVR (<100 mL)		Group 2 Elevated PVR (≥100 mL)		p
	Mean ± SD/n-%	Median	Mean ± SD/n-%	Median	
Max flow rate	26.7±12.7	24	30.3±11.8	28	**0.002**
Voiding volume	335.3±171.2	318	457.6±226.2	411	**<0.001**
Max bladder capacity	429±84.7	437	503.5±67	500	**<0.001**
Voiding efficiency vol/capacity*100	80.4±44.1	76	92.5±47.4	87	**0.011**

PVR: Postvoiding residual urine, SD: Standart Deviation

Table 3. Logistic regression analysis of voiding parameters though post-voidal residual urine in patients with overactive bladder

	Univariate model		
	OR	95% CI	p
Max flow rate	1.03	1.01-1.05	**0.013**
Voiding volume	1.00	1.00-1.00	**<0.001**
Max bladder capacity	1.01	1.01-1.02	**<0.001**
Voiding efficiency vol/capacity*100	1.01	1.00-1.01	**0.030**
First sensation volume	1.00	1.00-1.00	0.671
Detrusor overactivity	0.78	0.49-1.23	0.282
Valsalva leak point pressure	1.01	1.01-1.02	**<0.001**
Vesical pressure at Q_{max}	0.98	0.97-1.00	0.051
Abdominal pressure at Q_{max}	0.98	0.97-1.00	**0.025**
Detrusor pressure at Q_{max}	0.99	0.97-1.00	**0.048**

OR: Odds ratio, CI: Confidence interval

observed higher Q_{max} detrusor pressures than were found in our study. We hypothesized that the difference might be explained by the age differences of the study samples. The mean age in the present study was 71 years, whereas in Jeong et al.[16] the mean age was 58.9 years. It has been established that bladder and uretral profiles change with age. Women who had voided less than 100 mL (n=18) were excluded from the present study because the minimum voided volume for reliable urine flow rate in persons aged 56-80 years is 100 mL[17].

PVR volume can be measured using uroflowmetry or pressure-flow studies. These tests are non-invasive. Previous reports reflected a debate regarding the measurement of residual urine volume. Specifically, when voided volume was greater than 300 mL, the mean PVR volumes were not significantly different between non-invasive uroflowmetries and pressure flow studies[18]. Therefore, further studies on the differences in PVR volume in relation to voided volume and type of testing are required. Other limitations of the current study are related to its retrospective design. We did not use a bladder diary data to examine the women's naturally voided volume.

Conclusion

Patients with OAB with elevated PVR volumes showed increased maximum bladder capacity, but DO was not more prevalent in these women compared with women with normal PVR volumes.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.D., H.C, Concept: U.H., Design: M.E., Data Collection or Processing: Ç.H., Analysis or Interpretation: C.K., Literature Search: H.C., Writing: H.D.
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Depression and anxiety disorder in hyperemesis gravidarum: A prospective case-control study

Hiperemezis gravidarumlu kadınlarda depresyon ve anksiyete bozukluğu: Prospektif olgu kontrol çalışması

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Abstract

Objective: To assess the anxiety and depression status of women with hyperemesis gravidarum (HG); the risk factors for developing both depression and anxiety in women with HG were evaluated.

Materials and Methods: A total of 200 women, 100 diagnosed as having HG before the 20th week of gestation at a tertiary referral center and 100 gestational-age-matched controls were enrolled. The socio-demographic data and the depression and anxiety scores, as assessed using the Beck depression and anxiety inventory were compared between the two groups.

Results: The median depression and anxiety scores were significantly higher in the HG group compared with controls (19.5 vs. 9.0 and 22.0 vs. 10.0). Women with HG have the highest relative risks for moderate depression and severe anxiety [relative risk (RR): 16.88 and RR: 20.50, respectively]. In the univariate analysis, having HG, low education level, low income and poor social relationships were significant predictors of depression and having HG. Moreover, poor social relationships significantly predicted the presence of anxiety disorder. However, having HG and poor social relationships were found as the only independent predictors of both depression and anxiety. Patients with HG were 5.5 and 6.7 times more prone to having depression and anxiety disorder compared with controls, respectively.

Conclusion: Both depression and anxiety disorder were more frequent in women with HG who have weak family and social relationships, lower education and income levels. Therefore, the determination of the psychological status of women with HG should be an integral part of the evaluation.

Keywords: Hyperemesis gravidarum, pregnancy, depression, anxiety disorder

Öz

Amaç: Araştırmanın birincil amacı, hiperemezis gravidarum (HG) olan kadınların anksiyete ve depresyon durumlarını değerlendirmektir. Ayrıca, tüm gruptaki depresyon ve anksiyete için risk faktörlerini de değerlendirdik.

Gereç ve Yöntemler: Gebeliğin 20. haftasından önce üçüncü basamak bir merkezde HG teşhisi konan 100 kadın ve gebelik haftası eşleştirilen 100 kontrol olmak üzere toplam 200 kadın çalışmaya dahil edildi. Sosyo-demografik veriler ve Beck depresyon ve anksiyete envanteri ile değerlendirilen depresyon ve anksiyete skorları iki grup arasında karşılaştırıldı.

Bulgular: Medyan depresyon ve anksiyete skorları HG grubunda kontrol grubuna göre anlamlı derecede yüksekti (sırasıyla 19,5 ve 9,0 ve 22,0 ve 10,0). Orta sınıf depresyon ve şiddetli kaygı açısından HG'li kadınlar en yüksek relatif riske sahipti [relatif risk (RR): 16,88 ve RR: 20,50]. Tek değişkenli analizde; düşük eğitim düzeyi, düşük gelir ve zayıf sosyal ilişkiler depresyon ve HG'ye sahip olma yönündeki prediktif belirteçlerdir, zayıf sosyal ilişkiler anksiyete bozukluğunu önemli ölçüde öngörmüştür. Bununla birlikte, çok değişkenli analiz sonrası depresyon ve anksiyete bozukluğunun bağımsız öngördürücüleri olarak sadece HG ve zayıf sosyal ilişkiler bulunmuştur. HG hastalarının, kontrol grubuna göre depresyon ve anksiyete bozukluklarına sırasıyla 5,5 ve 6,7 kat daha fazla eğilimli olduğu bulundu.

Sonuç: Depresyon ve anksiyete bozukluğunun her ikisi de HG'li, zayıf aile ve sosyal ilişki, düşük eğitim ve düşük gelirli kadınlarda görülmüştür. Bu nedenle, özellikle düşük sosyo-ekonomik popülasyonlar için, hastaların psikolojik durumu, HG değerlendirmesinin vazgeçilmez bir parçası olmalıdır.

Anahtar Kelimeler: Hiperemezis gravidarum, gebelik, depresyon, anksiyete bozukluğu

PRECIS: We showed that having weak family and social relationships, lower socio-economic status played an important role in the development of depression and anxiety in pregnant women.

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Introduction

Nausea and vomiting during early pregnancy is very common and generally accepted as a part of normal physiology[1,2]. Hyperemesis gravidarum (HG) is a pregnancy condition characterized by severe nausea and vomiting starting before the 22nd week of gestation. Although it generally ends before the 16th week, HG may be severe in 2% of pregnant women, who require hospitalization[1,2]. Although there is no universally accepted criteria for the diagnosis, HG is characterized by persistent vomiting and nausea, weight loss of more than 5% of pre-pregnancy body weight, ketonuria, electrolyte abnormalities (hypokalemia), and dehydration (high urine specific gravity), resulting in the diminishment of the woman's quality of life and a significant contribution to health care costs and time lost from work due to persistent vomiting[3]. Despite its frequency, the etiopathogenesis of HG has not yet been clearly elucidated. Many theories based on endocrinologic and metabolic factors, gastrointestinal dysfunction, and immunologic, genetic, and psychological factors have been proposed[2-4]. Besides its physical symptoms such as dehydration and electrolyte imbalance, HG can also affect quality of life and the psychological state of pregnant women[5,6].

Depression is the most common psychological disease seen in women, and encountered in 14% to 48% of pregnant women[7-10]. Although recent studies demonstrated higher rates of depression and anxiety in pregnant women with HG, few studies have evaluated the predictive factors or cause-and-effect relation of these psychological disorders and pregnancy[11-15].

In this case-control study, the anxiety and depression disorder of pregnant women with HG was assessed, and we aimed to determine the risk factors for developing both depression and anxiety in pregnant women.

Materials and Methods

The study was conducted in a tertiary care center, Etlik Zübeyde Hanım Women's Health Training and Research Hospital from June 2013 to October 2013. The local ethics committee approved the study (approval number: 2013-165) and all participants gave written informed consent. The trial was performed in accordance with the Declaration of Helsinki. Pregnant women diagnosed as having HG before the 20th week of the current viable pregnancy and required hospitalization for intravenous fluid replacement were included in the study group. Persistent vomiting accompanied by weight loss exceeding 5% of pre-pregnancy body weight, an objective measure of acute starvation (usually large ketonuria on urine analysis), electrolyte abnormalities and acid-base disturbances, was diagnosed as HG. Gestational-age-matched controls were recruited from patients who came for routine antenatal care follow-ups. Patients who had multiple pregnancies, thyroid disease, prior psychiatric disease or conditions with elevated serum human chorionic gonadotropin levels such as gestational trophoblastic diseases and chromosomally abnormal fetus were excluded.

All participants were asked to complete a demographic and socioeconomic data collection form. In this form, patients assessed and rated their relations with other family members and society as strong or weak.

Anxiety and depression scores

The status of depression and anxiety was evaluated using the Beck Depression Inventory (BDI)-II and Beck Anxiety Inventory (BAI). The BDI includes a 21-question self-assessment scale and answers were scored from 0 to 3. BDI scores were grouped as follows: 0-9 as no depression, 10-16 as mild depression, 17-23 as moderate depression, and 24-63 as severe depression. Answers to each question in the BAI were scored from 0 to 3. The BAI scores were classified as follows: 0-7 as no anxiety, 8-15 as mild anxiety, 16-25 as moderate anxiety, and 24-63 as severe anxiety.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY). Univariate analyses to identify variables associated with anxiety and depression were investigated using appropriate statistical tests such as Student's t-test, the chi-square test and Mann-Whitney U tests. The Kruskal-Wallis test was used to compare the anxiety and depression scores between the two groups in terms of different variables. Bonferroni correction was used to test the significance with pairwise differences to adjust multiple comparisons. The association between ordinal variables was investigated, and correlation significance was calculated using the Spearman test. For multivariate analyses, possible factors identified were further entered into the logistic regression analysis to determine the independent predictors of anxiety and depression. A 5% type 1 error was used for the statistical significance.

Results

A total of 100 pregnant women with HG and 100 gestational-age-matched controls were included in the study. The socio-demographic data of all participants are listed in Table 1.

Using BDI, the median depression scores were significantly higher in the HG group compared with controls (19.5 vs. 9.0; $p<0.001$). Similarly, using BAI, the median anxiety scores were also higher in the HG group compared with controls (22.0 vs. 10.0; $p<0.001$). The percentage of pregnant women living on minimum wage was nearly double in the HG group compared with controls (61.9% vs. 38.0%; $p=0.01$). The education level was also significantly lower in the HG group compared with controls such that the percentage of women who graduated from at least high school among the HG and control groups were 40.4% and 59.6%, respectively ($p=0.005$). Pregnant women who defined their relationship with their families as "weak" had a higher percentage of HG than those who defined them as "strong" (69% vs. 31.0%; $p=0.027$). Similarly, women who had weak social relations

were also more prone to having HG (63.6% vs. 36.4% p=0.041).

When all other possible contributing factors were matched, patients in the HG group had a higher risk of having depression or anxiety (mild, moderate or severe) compared with controls (Table 2). The relative risk of women with HG was highest for moderate depression and severe anxiety (16.88 vs. 20.50).

Data of all participants were analyzed to determine the risk factors for depression and anxiety. HG, lower educational status, lower income, and poor social relationships were the significant risk factors for depression and HG, and poor social relationships was a significant risk factor for anxiety in the entire pregnant population (Table 3). Additionally, HG and poor social relationships were independent risk factors for both depression and anxiety in the entire cohort. Patients with HG were 5.5 times more prone to having depression and 6.7 times more prone to having anxiety compared with the matched controls. Additionally, risks of having depression and anxiety in pregnant women with poor social relationships were 2.8 and 5.6 times higher, respectively, when compared with controls (Table 4).

Discussion

The psychological theory for describing the pathogenesis of HG suggests that either the presence of conversion or somatization disorder, or the exaggerated response of a patient to stress may cause HG[16]. Although nausea and vomiting during pregnancy were more commonly seen in dependent, hysterical, depressive, and anxious women, severe and persistent vomiting itself might also cause the psychological problems in patients[17-22].

Table 1. Socio-demographic characteristics of the hyperemesis and control group

	HG[1] (n=100)	Control (n=100)	p value
Age (years)	26.0 (9.0)	25.0 (6.0)	0.254
Age at marriage (years)	21.0 (4.0)	20.0 (4.0)	0.741
Gravidity	2.0 (2.0)	2.0 (2.0)	0.859
Parity	1.0 (1.0)	1.0 (1.0)	0.775
Paid employment	21 (21%)	16 (16%)	0.363
Low income[2]	60 (60%)	37 (37%)	0.001
High school graduate	42 (42%)	62 (62%)	0.005
Poor relationships	20 (20%)	9 (9%)	0.027
Poor social relationships	28 (28%)	16 (16%)	0.041
Depression score	19.5 (11.0)	9.0 (8.8)	<0.001
Anxiety score	22.0 (14.5)	10.0 (11.8)	<0.001

Data presented as median (interquartile) and number (percentage)
[1]HG: Hyperemesis gravidarum
[2]Monthly income of less than 1070 Turkish Liras (approximately USD 480)

Table 2a. The relative risk of depression (a) or anxiety (b) in the hyperemesis and control groups

HG | control[1]	Odds ratio	95% CI[2] Min; Max	p value
Mild depression	2.53	1.14; 5.60	0.022
Moderate depression	16.88	7.18; 39.66	<0.001
Severe depression	3.86	1.21; 12.28	0.022

[1]Hyperemesis gravidarum group compared to controls, [2]Confidence interval
HG: Hyperemesis gravidarum, Min-Max: Minimum-Maximum, CI: Confidence interval

Table 2b. The relative risk of anxiety (b) in the hyperemesis and control groups

HG[1] | control	Odds ratio	95% CI[2] Min; Max	p value
Mild anxiety	2.62	1.05; 6.56	0.039
Moderate anxiety	9.11	3.64; 22.79	<0.001
Severe anxiety	20.50	7.16; 58.72	<0.001

[1]Hyperemesis gravidarum group compared to controls, [2]Confidence interval
HG: Hyperemesis gravidarum, Min-Max: Minimum-Maximum, CI: Confidence interval

In this study, we investigated the possible effects of many socio-demographic parameters on HG. Among these parameters, some (age, parity, previous miscarriage, age of marriage and working status) were found not to be related, whereas others (income level, education level, social and family relations) were found related. Anxiety and depression were significantly more common and more severe in the HG group than in the controls. Similar to Hizli et al.[15], we also found the incidence of depression as

Table 3. Parameters tested to be risk factors for depression or anxiety in early pregnancy: univariate analysis

Parameters		Incidence of depression	p value	Incidence of anxiety	p value
Age	Adolescent[1]	33.3%	0.999	33.3%	0.499
	Adult	35.1%		24.5%	
Parity	Nulliparous	38.9%	0.387	29.2%	0.307
	Multiparous	32.8%		22.7%	
Previous miscarriage	Yes	36.3%	0.459	27.9%	0.619
	No	30.2%		24.2%	
Age at marriage	Child marriage[2]	41.2%	0.577	35.3%	0.378
	Adult marriage	34.4%		24.0%	
Status of employment	Housewife	36.2%	0.457	25.2%	0.916
	Employee	29.7%		24.3%	
Income level	Lower than MW[3]	44.7%	0.003	22.7%	0.462
	Higher than MW	24.7%		27.2%	
Education level	Elementary school	43.3%	0.011	26.9%	0.513
	High school	26.0%		22.9%	
Family relationship	Poor	36.3%	0.365	26.9%	0.132
	Good	27.6%		13.8%	
Social relationship	Poor	39.7%	0.008	30.1%	0.002
	Good	18.2%		6.8%	
Hyperemesis gravidarum	Not present	16.0%	<0.001	9.0%	<0.001
	Present	54.0%		41.0%	

[1]Patient ≥19 years of age, [2]Formal marriage or informal union before age 18 years
MW[3]: Minimal wage

Table 4. Relative risk ratios of various parameters for depression and anxiety: multivariate analysis

	[1]RR	[2]95% CI (upper-lower)	p value
Risk factors for depression			
Low income	1.615	0.800-3.258	0.181
Lower education	1.361	0.673-2.752	0.390
Poor family relationship	1.934	0.637-5.869	0.244
Poor social relationship	2.842	1.037-7.791	0.042
HG	5.505	2.755-10.998	<0.001
Risk factors for anxiety disorder			
Poor social relationship	5.689	1.449-22.340	0.013
Poor family relationship	1.351	0.354-5.164	0.660
HG	6.704	2.974-15.111	<0.001

[1]Predictive relative risk calculated with odds ratio, [2]95% CI: Confidence interval, RR: Relative risk, HG: Hyperemesis gravidarum

84% in the HG group. This high incidence of depression might be attributed to the completion of questionnaires on the first day of hospitalization and high percentage of patients with low income. In the univariate analysis, patients with low income, low educational status, and poor social relationships had increased risk for having depression in the current study. An alternative view is that HG is an independent variable that increases the relative risk of all stages of anxiety and depression. The most dramatic effect of HG was seen on the development of severe anxiety. A woman with HG was 20.5 times more prone to having severe anxiety compared with healthy pregnant woman in the present study. These findings are consistent with some previous studies; however; some other studies could not demonstrate an association between severity of HG and depression[19-21].

Predictive factors for developing anxiety disorder and depression in pregnant women with HG were also investigated in a few studies[11,14,17]. Tan et al.[14] investigated the incidence and risk factors for developing anxiety and depression in a cohort of 209 women with HG in early pregnancy. HG was found to be the only independent risk factor for developing anxiety, and the only independent protective factor for developing depression was the history of miscarriage. In 2012, Hizli et al.[15] evaluated the impact of HG and socio-demographic variables on depression in Turkish patients during pregnancy. They used the BDI-II and reported the incidence of depression as 80% in the HG group and 5% in the control group. They also stated that the presence of HG was the most important predictor of depression, and additionally, young maternal age and poor family interaction were other weaker contributors. Recently, Annagür et al.[11] investigated the association between HG and eating attitudes on anxiety and depression symptoms in 48 women with HG and 44 controls. The authors concluded that HG was associated with symptoms of anxiety and depression but not with eating attitudes.

Study Limitations

The strengths of the study are the use of objective inclusion criteria and a relatively high number of patients, and the limitations are the use of psychiatrics test for depression instead of examinations by a psychiatrist, and the matching process between the study and control group was insufficient.

Conclusion

In this study, we showed that both depression and anxiety disorders were more common and severe in patients with HG compared with controls. In addition, having weak family and social relationships and lower socioeconomic status also played an important role in developing depression and anxiety in pregnant women. Therefore, the psychological state of patients should also be noticed during the evaluation for physical health of women with HG.

Ethics

Ethics Committee Approval: The study was approved by Etlik Zübeyde Hanım Women's Health Teaching and Research Hospital Ethics Committee (approval number and date: 2013-165/27.05.2013).

Informed Consent: A consent form was completed by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Endothelial cell leptin receptors, leptin and interleukin-8 in the pathogenesis of preeclampsia: An *in-vitro* study

Preeklampsi patogenezinde endotelyal hücre leptin reseptörleri, leptin ve interlökin-8: İn-vitro çalışma

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Abstract

Objective: Increased leptin hormone and leptin receptor may enhance the generation of proinflammatory cytokines by endothelial cells and lead to endothelial dysfunction. This study assessed the umbilical cord endothelial leptin receptor levels in preeclampsia and investigated the effect of leptin on endothelial interleukin-8 (IL-8) production.

Materials and Methods: The association between IL-8 levels with leptin stimulation was investigated in leptin-treated human endothelial cells. Endothelial cell leptin receptor levels were evaluated using immunohistochemistry staining, and endothelial IL-8 protein expression by Western blot analysis. Data are presented as mean ± standard error of the mean (SEM). Statistical significance was analyzed using Student's t-test or Mann-Whitney U test and one-way analysis of variance.

Results: Leptin receptor immunoreactivity increased significantly in umbilical cord venous and arterial endothelial cells in normal pregnancy (n=12) compared with preeclampsia (n=7) endothelial cells. The corresponding preeclampsia versus control histologic scores (mean ± SEM) were 67.9±8.8 vs. 127.6±23.1, (p=0.011) for the leptin receptor and 55.4±8,0 vs. 93.7±17.1 (p=0.035), respectively, for the vein endothelial cells. Leptin treatment significantly increased IL-8 protein levels (control vs. 100 and 1000 ng/mL, p=0.003).

Conclusion: The findings of increased umbilical cord endothelial leptin receptor levels in preeclampsia and increased endothelial IL-8 expression with exposure to higher leptin concentrations may indicate the contribution of leptin to endothelial dysfunction and increased neutrophil-endothelial interaction, which are significant pathophysiologic features of preeclampsia.

Keywords: Preeclampsia, endothelial cell, umbilical cord, leptin, leptin receptor, interleukin-8

Öz

Amaç: Dolaşımda artan leptin ve leptin reseptörü, endotel hücrelerinde proenflamatuvar sitokinlerin üretimini artırabilir ve endotel disfonksiyonuna neden olabilir. Bu çalışmada, preeklampside umbilikal kord endotelyal leptin reseptör düzeyleri değerlendirildi. Ayrıca leptinin endotelyal interlökin-8 (IL-8) üretimi üzerindeki etkisi araştırıldı.

Gereç ve Yöntemler: Endotel hücre leptin reseptör seviyesi immünohistokimyasal boyama ve endotelyal IL-8 protein ekspresyonu Western blot analizi yöntemleri kullanılarak değerlendirildi. Veriler ortalama ± standart hata (SEM) olarak sunuldu. İstatistiksel fark Student t-testi veya Mann-Whitney U testi ve tek yönlü varyans analizi kullanılarak analiz edildi.

Bulgular: Normal gebeliğe kıyasla (n=12), preeklampside (n=7), umbilikal kord venöz ve arteriyal endotel hücre leptin reseptör immünoreaktivitesi, anlamlı olarak artmış bulundu. Kontrollerde ve preeklampsi leptin reseptörleri için histolojik skorlar (ortalama ± SEM); umbilikal arter endotel hücrelerinde sırasıyla 67,9±8,8 ve 127,6±23,1 (p=0,011) iken, umbilikal ven endotel hücrelerinde sırasıyla 55,4±8,0 ve 93,7±17,1 (p=0,035) olarak saptandı. Yüksek leptin seviyelerine (100 ve 1000 ng/mL) maruz kalan endotel hücrelerinde, kontrole kıyasla, IL-8 düzeylerinin anlamlı olarak arttığı gözlendi (p=0,003).

Sonuç: Preeklampside umbilikal kord endotelyal leptin reseptör düzeylerinin artmış olması ve yüksek leptin konsantrasyonlarının endotelyal IL-8 ekpresyonunu uyarması; leptinin, preeklampsinin belirgin patofizyolojik özellikleri olan endotel disfonksiyonu ile artmış nötrofil-endotel etkileşimine katkısı olabileceğini göstermektedir.

Anahtar Kelimeler: Preeklampsi, endotelyal hücre, umbilikal kord, leptin, leptin reseptörü, interlökin-8

PRECIS: This study identifies, higher levels of leptin expressions in umbilical cord from preeclampsia placenta, suggesting that provocation of interleukin-8 secretion by leptin in endothelial cells may contribute to preeclampsia-related inflammation.

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Introduction

Preeclampsia (PE) is a multisystem disorder. Hypertension, proteinuria, and pathologic edema in pregnancy are the classic clinical manifestations. Worldwide, approximately 5-7% of primigravid women develop PE in pregnancy[1]. Many studies implied that the fundamental pathophysiologic abnormality is endothelial dysfunction associated with exaggerated inflammation and immunologic reaction[2-4]. In the pathogenesis of PE, activation of neutrophils, monocytes, and natural killer cells initiate inflammation, resulting in endothelial destruction in the pathogenesis of PE[4]. The damaged endothelium produces various chemokines, one of which is interleukin (IL)-8[5].

Leptin was initially defined as an adipocyte hormone that controls energy balance, reproductive functions, and immune reactions in the body[6]. Mounting data imply that leptin is a novel proinflammatory adipocyte-originated element that manages the cytokine pathway by connecting the immune and inflammatory system[7,8]. The plasma leptin concentrations in patients with PE are considerably higher than those in normal patients[9]. Moreover, recent reports revealed that leptin showed vital roles in diverse physiologic processes including angiogenesis and arterial blood pressure regulation[9].

IL-8 has been reported to activate chemotactic migration, proliferation, and survival of vascular endothelial cells; induce the expressions of vascular endothelial growth factor (VEGF) and VEGF receptor[10], and regulate pathologic angiogenesis[11]. Was first purified as a potent neutrophil chemotactic factor[12]. IL-8 is also characterized as a proinflammatory cytokine. Increased IL-8 levels have been described in various inflammatory diseases[13]. Many inflammatory cells, such as monocytes, lymphocytes, and mast cells, release IL-8 that is accumulated inside endothelial cells. IL-8 and its receptors C-X-C chemokine receptor 1-2 have been detected in endothelial cells[14]. Moreover, human placental tissue produces IL-8 during pregnancy. Previous studies showed a robust correlation between IL-8 levels and the severity of PE[5,15]. Neutrophil activation results in progressive damage to endothelial cells[16]. The present study aimed to investigate umbilical cord (UC) leptin receptor (LEPR) levels in PE and to examine the direct effect of leptin on IL-8 production by human endometrial endothelial cells (HEECs) and human umbilical vein endothelial cells (HUVECs), thus to describe the function of leptin in PE.

Materials and Methods

Tissue collection

Serial paraffin sections of human placental UC specimens were obtained from the University of South Florida under the protocol approved by the Ethics and Human Investigation Committees of the University of South Florida (approvel number: 00015578). Written and verbal informed consents were obtained from each patient. All of the samples were grouped according to clinical diagnosis: UC control (n=12) or PE (n=7). For *in vitro* studies, previously frozen HEECs (n=2) and HUVEC (n=1) from normal women undergoing hysterectomy (laparoscopy or laparotomy) or normal delivery were thawed and grown to confluence, as previously described[17].

Immunohistochemistry

Collected PE and normal patient UC paraffin blocks were cut into 5 µm sections that were then put into a heater to incubate overnight at 56 °C. The slides were deparaffinized in xylene (x3) for 20 min., followed by 100, 90, 80, and 70% alcohol x1 for 10 min. per gradient. Following deparaffinization, the slides were heated in 10 mM citrate buffer (pH 6.0) for 3x5 min in a microwave oven for antigen retrieval. The slides were then immersed in 3% hydrogen peroxide (in 1:1 v/v methanol/distilled water) for 12 min. to quench endogenous peroxidase activity. After washing with tris-buffered saline (TBS); (pH: 7.4) (x3) for 5 min., the slides were incubated in a humidified chamber with 5% blocking normal goat serum (Vector Labs, Burlingame, CA) for 30 min at room temperature (RT) in TBS. Excess serum was emptied, and then the slides were incubated overnight with a primary rabbit polyclonal anti-LEPR antibody (1:60; Santa Cruz Biotechnology, Dallas, TX) in 1% normal goat serum at 4 °C. Normal rabbit immunoglobulin G (IgG) (Vector Labs) isotypes were used for negative controls at the equal primary antibody concentrations. The slides were rinsed (x3) for 5 min. with TBS, and then biotinylated anti-rabbit IgG (Vector Labs) was used at a 1:400 dilution for 30 min. at RT. The antigen-antibody complex was identified using an avidin-biotin-peroxidase kit (Vector Labs) for 30 min. at RT. 3.3´-Diaminobenzidine tetrahydrochloride dihydrate (Vector Labs) was added as the chromogen to visualize immunoreactivity for 90 seconds. The slides were then counterstained with hematoxylin and mounted. Immunoreactive LEPR levels were semi-quantitatively assessed using the subsequent intensity categories: 0, no staining; 1^+, weak but visible staining; 2^+, moderate or distinct staining; and 3^+, strong staining. As described previously[18], for each tissue, a histologic score (HSCORE) was derived by adding the percentages of cells that were stained at each intensity category and then multiplying that value by the weighted intensity of the staining using the formula HSCORE=Σ Pi (i + l), where i represents the intensity scores, and Pi is the corresponding percentage of the cells. In each slide, three randomly selected areas were assessed under a light microscope (x40 magnification), and the percentage of the cells at each intensity within these regions was evaluated at different times by two blinded researchers. The average HSCORE of the two examiners was used. Human endometrial endothelial cell and human umbilical vein endothelial cell isolation and experimental treatment with leptin. Frozen primary HEECs (n=2) and HUVECs (n=1) were derived from banked samples. The samples had been isolated and categorized as previously described[17] from endometrial specimens obtained from reproductive-age women undergoing hysterectomy

(laparoscopy or laparotomy) and UC vein specimens obtained from the delivery of a normal pregnant woman. Written informed consent for sample retrieval was obtained from Yale University Faculty of Medicine, Human Investigation Committee and approved by the University of South Florida. Aliquots of frozen primary HEECs and HUVECs were thawed and grown to confluence in basal medium (BM), and a phenol red-free 1:1 v/v Dulbecco's Modified Eagle Medium/Ham's F-12 (Gibco, Grand Island, NY) mixture, containing 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL Fungizone complex (Gibco) supplemented with 10% charcoal-stripped calf serum (Gibco). At ~80% confluence, HEECs and HUVECs were transferred to 6-well plates at a density of $150x10^3$ cells/well, for the corresponding treatments, as designated by each experimental condition. Confluent HEECs and HUVECs were incubated in parallel in BM with 0.1% ethanol (vehicle control) or leptin (0.1, 1.0, 10, 100 and 1000 ng/mL leptin, respectively). After incubation for 24 h, HEECs and HUVECs were rinsed with ice-cold 1x phosphate buffered saline and stored at -80 °C until used for immunoblotting analysis to measure IL-8 total protein levels.

Immunoblot analysis

Total protein was extracted using a cell extraction buffer (BioSource International, Camarillo, CA) containing 3 mM phenylmethylsulfonyl fluoride and a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). The protein level was determined using a detergent-compatible protein assay (Bio-Rad, Hercules, CA). Samples (40 µg) were loaded on 10% Tris-hydrochloric acid-ready gels (Bio-Rad), electrophoretically separated, and electroblotted onto a nitrocellulose membrane (Bio-Rad). The membrane was blocked with 5% non-fat milk powder in TBS containing 0.1% Tween 20 (TBS-T) for 1 h to reduce any non-specific antibody binding. Subsequently, the membrane was incubated overnight with a monoclonal mouse IgG1 clone primary antibody against IL-8 (1:800 R&D Systems, Inc., Minneapolis, MN) in 5% non-fat milk powder in TBS-T. The membrane was then rinsed several times with 1x TBS-T for 1 h and incubated with horseradish peroxidase-conjugated anti-rabbit IgG (Vector Labs) in TBS-T. Following several washes, IL-8 was visualized through light emission from the film (Denville Scientific, Holliston, MA) with enhanced chemiluminescence substrate (Thermo Scientific, Rockford, IL). Band intensities were quantified using computer densitometry analysis (Image J, National Institutes of Health, Bethesda, MD).

Statistical Analysis

Data from immunohistochemistry and Western blot analysis that were normally distributed, according to the Kolmogorov-Smirnov test, were compared using Student's t-test or one-way analysis of variance, followed by the post hoc Holm-Sidak test. Immunohistochemistry and Western blot analysis data that were not normally distributed were analyzed using the Kruskal-Wallis nonparametric ANOVA-by-Ranks test, followed by the post hoc Student-Newman-Keuls test. Statistical calculations were performed using Sigmaplot 13 for Windows (Jandel Scientific Corp., San Rafael, CA). Statistical significance was considered as $p<0.05$.

Results

Immunohistochemistry of human placental umbilical cord specimens evaluating leptin receptor immunostaining in the control vs. preeclamptic groups

LEPR immunostaining was detected in the cytoplasm and nucleus of the UC artery and vein endothelial cells. LEPR HSCOREs were significantly different between the control vs. PE specimens in the sectioned UC arterial endothelial cells [mean ± standard error of the mean (SEM): 67.9±8.868 vs. 127.6±23.1; p=0.011, respectively] (Figure 1) and UC vein endothelial cells (mean ± SEM: 55.4±8.043 vs. 93.7±17.15; p=0.035, respectively). LEPR immunostaining was moderate in the PE specimens (n=7) (Figure 1a-d), whereas the control UC endothelial cells (n=12) displayed weak immunostaining (Figure 1e-h). There were no statistically significant differences in maternal age, gestational age (GA), body mass index (BMI) at delivery, and baby weight between the control and PE groups (Table 1).

Effect of leptin on interleukin-8 protein expression in cultured human endometrial endothelial cells and human umbilical vein endothelial cells

Experimental incubations were followed by immunoblotting of the cell extracts to establish the functional regulation of leptin on IL-8 protein expression in the primary cultures of HEECs and HUVECs. Representative immunoblotting (Figure 2a) and the accompanying graphs (Figure 2b) indicated that IL-8 protein level was significantly increased by 1000 ng/mL leptin and 100

Table 1. Demographic data of the control and preeclamptic groups from which umbilical cord specimens were obtained

	Control (n=12)	Preeclampsia (n=7)	p value
Maternal age (years ± SD)	32.7±2.265	28.625±1.625	0.247
Gestational age (weeks ± SD)	37.50±0.44	37.14±0.36	0.152
BMI at delivery (kg/m^2 ± SD)	30.50±2.72	34.00±4.14	1.62
Birth weight of the baby (gr ± SD)	3534.16±408.41	2670.62±621.43	0.056

SD: Standart deviation, BMI: Body mass index

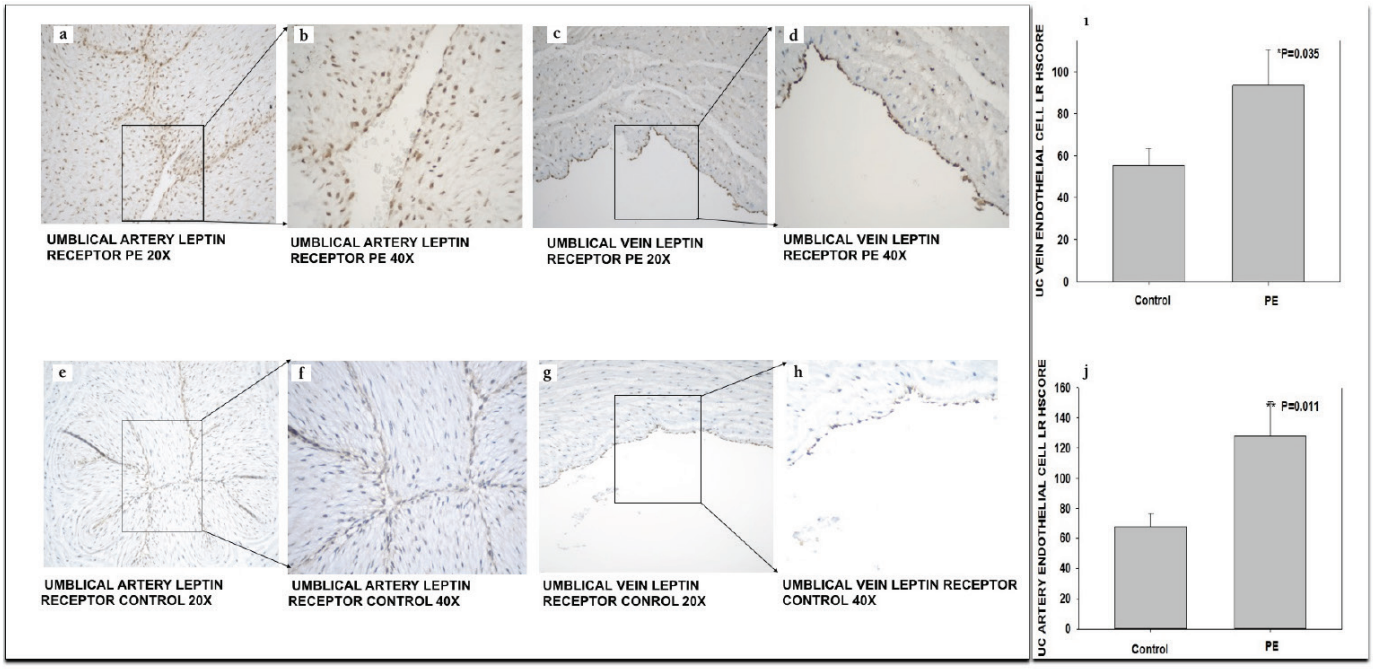

Figure 1. Leptin receptor immunoreactivity in umbilical cord arterial and venous sections from gestational age-matched preeclamptic and normal pregnancies. Representative micrographs of immunohistochemical staining for leptin receptor in preeclampsia (n=7) (A, B, C, D) and normal pregnancy (n=12) (E, F, G, H). Graphs represent the histologic score analysis of leptin receptor immunostaining in umbilical cord vein (I) and umbilical cord artery (J) endothelial cells expressed as mean ± standard error of the mean

**p=0.035; endothelial cells of the umbilical vein, preeclampsia vs. normal pregnancy*

***p=0.011; endothelial cells of the umbilical artery, preeclampsia vs. normal pregnancy*

PE: Preeclampsia

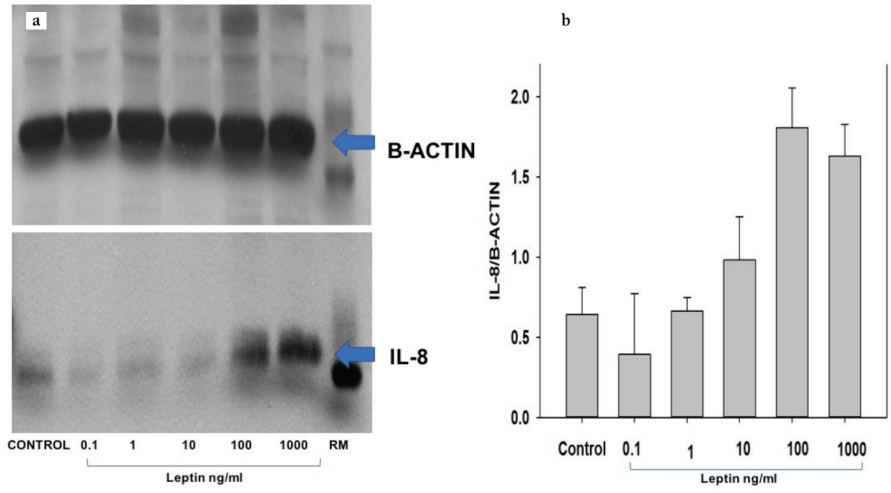

Figure 2. Leptin stimulates interleukin-8 expression in endothelial cells. Representative immunoblotting in human endometrial endothelial cells (n=2) and human umbilical vein endothelial cells (n=1) cultures treated with leptin for 24 hours. Western blot analysis demonstrating the effect of leptin on interleukin-8 levels in human endometrial endothelial cells and human umbilical vein endothelial cells. Confluent human endometrial endothelial cells and human umbilical vein endothelial cells cultures were treated with vehicle (control), 0.1 1, 10, 100 and l000 ng/mL leptin, respectively, for 24 hours, to evaluate the effect of leptin. Immunoblot bands for interleukin-8 were quantified using Image J. Bars represent mean ± standard error of the mean (n=3)

**p<0.05; both for 100 and 1000 ng/mL leptin vs. control.*

Data are representative of three independent experiments

IL: Interleukin

ng/mL compared with the control (p=0.003). Conversely, 0.1, 1, and 10.0 ng/mL leptin had not statistically significant effect on IL-8 levels when added to the culture medium (p>0.05) (Figure 2a, b). Incubation with 100 and 1000 ng/mL leptin induced greater IL-8 protein level vs. the control, 0.1 and 1 ng/mL leptin (Figure 2a). Compared with the control, 0.1, 1, and 10 ng/mL leptin showed no significant change in the basal IL-8 protein expression in HUVECs and HEECs.

Discussion

Several studies illustrated the potential role of cytokines, chemokines, and their receptors in the development and progression of PE[19-21]. Pregnancy complications associated with PE are major causes of materno-fetal morbidity and mortality, but their pathogenesis remains unclear[22]. Despite significant development in the management of PE, it still constitutes an unsolved health problem in pregnancy[23]. The current study examined the molecular mechanism of leptin-mediated inflammation in PE. The data in this study imply that leptin/LEPR interaction stimulated IL-8 production in endothelial cells, consequently promoting neutrophil chemotaxis and PE progression. Therefore, a view was instigated to connect leptin concentrations and IL-8 level in endothelial cells. The structure and function of endothelial cells are crucial to the preservation of arterial and vein vessel wall homeostasis, as well as immune cells migration[24]. Damaged endothelial cells, increasing inflammatory cell recruitment, and high concentrations of inflammatory agents in the plasma of pregnant patients are responsible for the pathogenesis of PE[13,24]. Impaired placental function and placental vascular disorders result in the occurrence of poor perinatal outcome[25]. Leptin is produced by cytotrophoblasts and syncytiotrophoblasts in the human placenta and adipose tissue, which is then secreted into the circulation, where it exerts its effects via interaction with the LEPR. The effect of leptin on endothelial cells has attracted particular attention[20,26]. LEPRs are expressed in many normal tissues, but also in pathologic tissues generally associated with obesity and abnormal energy balance[27]. Immunohistochemically, endothelial cells, syncytiotrophoblasts, and cytotrophoblasts were stained with leptin[28]. The present study demonstrated that the LEPR expression in UC artery and vein endothelial cells was augmented in PE and localized to the cytoplasm and nuclei of endothelial cells. Significant increases in the concentration and amounts of LEPR-positive endothelial cells, as implied by HSCORES in PE vs. normal pregnancy suggests a function for LEPR in the inflammatory pathway of PE. A previous study demonstrated a high level of the leptin gene expression in microarray investigations in PE[29]. In this regard, the current study was designed to elucidate the possible correlation between UC artery and vein LEPR levels and PE and its functional effects on endothelial cells. The study data can also be considered as indirect evidence for LEPR and its contribution to PE, and the functional results of leptin on endothelial cells. The data of the study can also be considered as indirect evidence for the contribution of LEPR to PE. Leptin possibly has an effect on the regulation of arterial blood pressure in pregnant women, as indicated by the direct relationship between plasma leptin concentrations and mean arterial blood pressure[30]. Furthermore, dysfunctions of leptin metabolism or regulation in the placental unit and plasma are enhanced in pregnancies complicated with various abnormalities such as intrauterine growth restriction, gestational diabetes mellitus, and PE. Leptin synthesis and secretion have been shown as positively correlated with BMI and GA[31-33]. In contrast, in this study, BMI, GA, maternal age, and birth weight of the baby were not significantly different between the control and PE groups. Mise et al.[34] stated that leptin messenger RNA (mRNA) levels in severe PE were significantly higher than in those with mild PE, also that placental leptin mRNA levels were generally similar to plasma leptin concentrations in women with PE compared with GA-matched healthy pregnant women. Higher concentrations of UC plasma leptin have been revealed in infants of mothers with PE than in a GA-, sex-, and infant ponderal index-matched control group[35]. Compromised placental blood distribution causes chronic disturbance of nutrient resource and ultimately results in intrauterine growth restriction[36]. Impaired placental perfusion also creates a depressed oxygen source at the placental level, which subsequently enhances leptin gene expression in the placental unit[21]. It is likely that the high leptin concentrations in maternal plasma may augment hypertension because leptin provokes endothelial dysfunction and hypertension via aldosterone-related mechanisms and milieu in gestations complicated by intrauterine growth restriction[37]. PE is concomitant with shallow trophoblastic invasion into the endometrial layer, which leads to poor placental perfusion and augmented fetal and maternal plasma leptin levels that are considerably increased over the concentration of leptin specific to human gestation[38]. This exaggerated hyperleptinemia may be linked to a compensatory response to augment nutrient supply to the growing fetus[39]. The present study showed that although not significant, the LEPR level was slightly higher in the UC artery than the paired UC vein, especially in the preeclamptic group, suggesting that UC artery LEPR may be more functional than UC vein LEPR in PE. In a previous report, leptin levels were found to be higher in the UC vein than the UC artery[40]. One other study showed that the leptin concentrations were considerably higher in UC artery and UC vein than those in paired maternal plasma, implying that leptin is produced in placental trophoblastic cells and is secreted into the maternal blood circulation. Furthermore, plasma leptin concentrations in the UC vein were notably higher than those in the paired UC artery, suggesting that leptin is released from placental trophoblastic cells into the fetal blood circulation[41].

The increase in the proinflammatory chemoattractant cytokine levels in PE suggests an inflammatory basis for this disease. The proinflammatory and regulatory cytokine IL-8 has been discovered in endothelial cells. Prior studies demonstrated upregulation of IL-8 protein levels in PE. IL-8 is regulated by neutrophil and monocyte chemotaxis regarding the inflammation site and stimulated inflammatory response[42]. IL-8 is secreted by some cell types, including endothelial cells, monocytes, macrophages. Neutrophils, and fibroblasts. This study investigated UC LEPR levels in PE and the relationship of IL-8 expression in HEECs and HUVECs with leptin at different concentrations in an attempt to explain the significant association between leptin and IL-8 in patients with PE. Increasing placental endothelial IL-8 production may contribute to the improvement of placental endothelial pathology in PE. There was a dose-dependent progressive connection between increasing leptin level and endothelial IL-8 protein expression. Exposing endothelial cells to a high leptin concentration plays a role in leukocyte migration into the placental area and in the management of the tissue-specific modifications related with the leukocyte extravasation. A prior study showed that circulating plasma IL-8 concentrations were elevated in women with PE compared with normal pregnant women[5,15]. These data indicate that IL-8 may have a critical role in endothelial cell proliferation and differentiation and regulating endothelial function. Human placental tissues constitutively produce IL-8 in pregnancy, and IL-8 secretion increases with progressing GA. IL-8 is critical for leukocyte recruitment[43]. Namely, the vascular endothelial cell layer acts as the gatekeeper for maternal immune rejection and immune cells. Similarly, increased IL-8 plasma levels in PE have been documented[15,44]. Moreover, leptin induced the production of IL-8 in human cartilage, fibroblasts, and M2 macrophages[45,46]. These previous data also support the role of leptin-induced IL-8 secretion in endothelial cells[46]. Additionally, the current study is the first to describe the association between leptin and IL-8 in HUVECs and HEECs. IL-8 influences early vascular remodeling by recruiting circulating neutrophil cells to the endothelial cells[24]. Understanding the mechanism for the increased IL-8 expression in HEECs and HUVECs of women in pregnancy may contribute to explaining the pathophysiology and development of PE. Speculatively, increased leptin production may exaggerate cytokine-induced destruction of endothelial cells in PE or overweight patients. Neutrophil-endothelial contact is a hallmark of vascular inflammation that results in endothelial injury/dysfunction[47]. Increasing IL-8 levels in endothelial cells probably contribute to enhanced neutrophil recruitment and cytokine production. Furthermore, neutrophil stimulation is amplified in inflammatory reactions in the maternal artery and vein blood circulation in PE. These observations reveal that there is a high rate of IL-8 production in PE, consistent with other studies[44]. However, further studies are required to investigate the role of leptin and LEPR in other inflammatory-related cellular mechanisms.

Collectively, these *in vivo* and *in vitro* results indicate that endothelial cells contribute to increased IL-8 concentrations in maternal and fetal circulation, as well as neutrophil recruitment. Leptin-associated augmented IL-8 secretion in endothelial cells is probably related with the development of PE. Thus, the increase in IL-8 level may potentiate leukocyte activation into the placental tissue under the effects of leptin. Consequently, the source and physiologic importance of IL-8 in the maternal and fetal circulation are noteworthy objectives of potential investigation. However, whether this phenomenon is a compensatory effect or amplified reaction to the severity of the PE remains enigmatic[48].

Conclusion

These observations provide direct evidence of the stimulation of IL-8 gene expression in endothelial cells *in vitro* by high leptin concentrations. In this manner, the increased leptin and LEPR may cause or contribute to increased IL-8 production, leading to increased neutrophil recruitment and endothelial destruction and consequently, increase cytokine expression in PE. This study may provide an *in vivo* basis for the application of an anti-human IL-8 antibody for the treatment of PE. Further studies researching the possible role of LEPR in normal and PE UC endothelial cells are needed to explore these possibilities and to support new insight into our understanding of the pathogenesis of PE.

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Ethics

Ethics Committee Approval: Serial paraffin sections of human placental UC specimens were obtained from the University of South Florida under the protocol approved by the Ethics and Human Investigation Committees of the University of South Florida (approval number: 00015578).

Informed Consent: Written and verbal informed consents were obtained from each patient.

Peer-review: External and internal peer-reviewed.

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

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The oxidative/anti-oxidative effects of sevoflurane on reproductive system of females: An experimental study

Dişilerin üreme sistemleri üzerinde sevofluranın oksidatif/anti-oksidatif etkileri: Deneysel çalışma

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Abstract

Objective: A permanent balance exists between the production and elimination of reactive oxygen species in all living organisms. The aim of this study was to evaluate the effects of sevoflurane possibly causing an imbalance in the equation of reactive oxygen species on the female rat reproductive system.
Materials and Methods: A total of 30 adult female Wistar-albino rats were placed into an anesthesia chamber to administer sevoflurane. Rats were randomly divided into six groups, each group consisting of five rats: the control group received 2 L/min O_2 18 min/day for seven days; the first group received 1 minimum alveolar concentration (MAC) of sevoflurane and 2 L/min O_2 18 min/day for seven days; the second group received 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for seven days with no treatment for the next seven days; the third group received 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for 14 days; the fourth group received 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for 14 days with no treatment for the next seven days; and the fifth group received 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for 14 days with no treatment for the next 14 days. Bilateral ovaries were subsequently removed for biochemical analysis of tissue anti-oxidative enzyme levels.
Results: Slight fluctuations were detected in mean nitric oxide, prostaglandin E2, prostaglandin F2-alpha, superoxide dismutase, glutathione peroxidase, malondialdehyde, alginate dialdehyde, and xanthine oxidase levels between the groups; however, the differences were not significant ($p>0.05$).
Conclusion: Sevoflurane has no effect on the activity of anti-oxidant systems in the rat ovary.
Keywords: Oxidative stress, nitric oxide, malondialdehyde, reproductive system

Öz

Amaç: Tüm yaşayan organizmalarda reaktif oksijen radikallerinin üretimi ve yıkımı arasında kalıcı bir denge mevcuttur. Bu çalışmanın amacı, dişi rat üreme sistemindeki dengenin bozulması üzerine sevofluranın olası etkilerini değerlendirmektir.
Gereç ve Yöntemler: Toplam 30 adet Wistar-albino rat sevofluran uygulamak için bir anestezi odasına yerleştirildi. Ratlar randomize olarak her biri beş rat içeren altı gruba bölündü: Kontrol grubu yedi gün boyunca 18 dakikada 2 L/dk O_2; birinci grup yedi gün boyunca 18 dakikada 1 minimum alveolar konsantrasyon (MAK) sevofluran ve 2 L/dk O_2; ikinci grup yedi gün boyunca 18 dakikada 1 MAK sevofluran ve 2 L/dk O_2 ve takip eden yedi gün boyunca herhangi bir tedavi almadı; üçüncü grup on dört gün boyunca 18 dakikada 1 MAK sevofluran ve 2 L/dk O_2; dördüncü grup on dört gün boyunca 18 dakikada 1 MAK sevofluran ve 2 L/dk O_2 ve takip eden yedi gün boyunca herhangi bir tedavi almadı; beşinci grup on dört gün boyunca 18 dakikada 1 MAK sevofluran ve 2 L/dk O_2 ve takip eden on dört gün boyunca herhangi bir tedavi almadı. İki taraflı overler doku anti-oksidatif enzim seviyelerinin biyokimyasal analizi için hızlı bir şekilde çıkarıldı.
Bulgular: Grupların ortalama nitrik oksit, protaglandin E2, prostaglandin F2-alfa, süperoksit dismutaz, glutatyon peroksidaz, malondialdehit, aljinat dialdehit ve ksantin oksidaz seviyeleri arasında hafif dalgalanmalar tespit edildi ancak bu değişiklikler anlamlı değildi ($p>0,05$).
Sonuç: Rat overlerinde sevofluranın anti-oksidatif sistemlerin aktivitesi üzerine herhangi bir etkisi yoktur.
Anahtar Kelimeler: Oksidatif stres, nitrik oksit, malondialdehit, üreme sistemi

PRECIS: Sevoflurane did not show any effect on the activity of anti-oxidant systems in the rat ovary.

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Introduction

Sevoflurane [2,2,2-trifluoro-1- (trifluoromethyl) ethyl fluoromethyl ether], which has a boiling point of 58.6 °C, a vapor pressure of 160 mm hemoglobin at 20 °C, and a blood-gas partition coefficient of 0.69 (approximately half of isoflurane), is pleasant-smelling and relatively non-irritating to the airways providing a high inhaled concentration without any adverse effects or irritation[1].

Oxidative stress is a condition as a consequence of an irregularity between the production and elimination of reactive oxygen species that are spontaneously generated during aerobic respiration and consumed endogenously. The tendency of the balance through free oxygen radicals can be deleterious for the sustainability of the life of a cell. Nitric oxide, one of the free radicals, plays a crucial role in the female reproduction system and manages the endometrial, myometrial, and microvasculatory tasks by paracrine functions.

Superoxide dismutase activity has been shown in the granulose and theca cells of the follicle, where glutathione peroxidase enzyme is localized in follicular fluid[2]. In contrast, with alginate dialdehyde, the oxidized form of alginate, there has been no study investigating the possible effects on female reproduction[3].

Furthermore, prostaglandin E2 and F2-alpha are autocrine and paracrine lipid mediators that are increased during the late secretory phase in which reactive oxygen species trigger the production of prostaglandin F2-alpha[2,3]. A study conducted by Yalçınkaya et al.[4] on the effects of follicular fluid nitric oxide, malondialdehyde, and reduced glutathione on *in vitro* fertilization outcomes demonstrated that malondialdehyde level was high in the follicular fluid of women with pregnancy, whereas nitric oxide was low. They also found that a positive correlation existed between malondialdehyde levels and the number of grade 1 embryos, and fertilization rates[4]. In this context, several drugs have been investigated for the production or the effects on the anti-oxidative enzyme systems of the body. The role of anesthetic agents is a very popular topic for researchers. Various studies conducted on the impacts of volatile anesthetics on the anti-oxidant system of different tissues showed controversial results. Sevoflurane decreases the intensity of oxidative stress and induced the activity of antioxidant defense mechanisms in erythrocytes[5-10]. Limited studies have been performed on the impacts of sevoflurane on reproductive tissues[8,9]. Therefore, the present study aimed to evaluate the impact of sevoflurane on the oxidant/anti-oxidant systems in the female reproductive tissue of rats.

Materials and Methods

After approval of the Animal Experiments Local Ethics Committee (2016-HADYEK-12), a total of 30 adult female Wistar-albino rats (90 days-old, 250-300 grams, all selected in the same period of estrus cycle as estrus by assessing vaginal smears) were obtained from the experimental medicine unit. Rats were housed in a room sustained at 20-24 °C with a 12-h light-dark cycle (lights on at 06:00 to 18:00) and constant humidity of 40-50%. All animals were kept in polycarbonate cages and given tap water ad libitum.

For sevoflurane exposure, rats were moved to a 40x50x60 cm anesthesia chamber, which had a connection with an anesthesia system (Prima SP Alpha, Penlon Limited, Oxon, UK). As previously described by Ceyhan et al.[11], two holes, one at the top left side of the chamber and the other at the upper right side of the chamber, were opened for anesthetic gas inlet and outlet. Animals were randomly separated into six groups, each group included five rats: the control group (C) was administered 2 L/min O_2 18 min/day for seven days; the first group (S1) was administered 1 minimum alveolar concentration (MAC) of sevoflurane and 2 L/min O_2 18 min/day for seven days; the second group (S2) was administered 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for seven days with no treatment for the next seven days; the third group (S3) was administered 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for 14 days; the fourth group (S4) was administered 1 MAC of sevoflurane and 2 L/min O_2 in 18 min/day for 14 days with no treatment for the next seven days; and the fifth group (S5) was administered 1 MAC of sevoflurane and 2 L/min O_2 18 min/day for 14 days with no treatment for the next 14 days. Animals were anesthetized by intraperitoneal injection of ketamine 90 mg/kg (Alfasan International B.V., Woerden, NL) with xylazine 10 mg/kg (Alfasan International B.V., Woerden, NL), and were killed by performing a cervical dislocation at the end of the 7th day in C and S1, the 14th day in S2 and S3, the 2nd day in S4, and the 28th day in S5. Bilateral ovaries were subsequently removed. The ovaries of each animal were placed on ice and then transferred to a -70 ˚C freezer where they remained frozen until biochemical analysis of tissue anti-oxidative enzyme levels.

Tissue nitric oxide level detection

Nitric oxide is a fast-eliminated molecule that is oxidized to nitrite and subsequently nitrate, which are used as the index parameters of nitric oxide production. The Griess reaction was performed for the detection of plasma nitrite and nitrate levels[12]. First, the protein fraction of the samples was removed using Somogyi reagent. After the total transformation of nitrite to nitrate using coppered cadmium granules, nitrite levels were calculated using spectrophotometry at 545 nm. A reaction curve was constructed with a pack of serial dilutions (10^{-8}-10^{-3} mol/L) of sodium nitrate. Outcomes were calculated as micromole per liter (micromol/L).

Tissue superoxide dismutase activity detection

Total (copper-zinc, manganese) superoxide dismutase activity was defined using the method previously described by Sun et al.[13] In brief, the method depends on the formation of nitro blue tetrazolium chloride reduction through the xanthine-xanthine oxidase system. After adding 1 mL ethanol/chloroform mixture (5/3, v/v) to the sample and centrifugation, superoxide

dismutase activity was evaluated in the ethanol phase of the sample. One unit of superoxide dismutase was determined as the enzyme intensity providing 50% inhibition in the nitro blue tetrazolium chloride reduction ratio. Superoxide dismutase activity was displayed as units per liter (U/L).

Tissue glutathione peroxidase activity detection

Glutathione peroxidase activity was calculated using the technique of Paglia and Valentine[14]. A chemical reaction was triggered by adding hydrogen peroxide in the mixture of sodium azide, nicotinamide adenine dinucleotide phosphate, glutathione reductase, and reduced glutathione. Thereafter, the absorbance of this solution was calculated using a spectrophotometer at 340 nm. Activity is presented as units per milliliter (U/mL).

Tissue xanthine oxidase activity determination

The measurement was performed using the method reported by Prajda and Weber.[15]. The activity of xanthine oxidase was calculated using the production of uric acid via xanthine through a spectrophotometry elevation at 293 nm. A functional curve was established using 10-50 mL concentrations of standard xanthine oxidase solutions (Sigma X-1875). One unit of activity was determined as one micromol of uric acid produced per minute (37 °C, pH 7.5), and presented as U/mL.

Tissue alginate dialdehyde activity detection

Tissue protein concentrations were measured using the technique previously described by Lowry et al.[16].

Prostaglandin E2 concentration

Prostaglandin E2 levels were measured using a prostaglandin E2 (514010) enzyme-linked immunosorbent assay (ELISA) (Cayman Chemical Company, Ann Arbor, MI, USA) kit as described in the manufacturer's instructions and presented as pg/mL.

Prostaglandin F2-alpha concentration

Prostaglandin F2-alpha levels were calculated using a prostaglandin F2-alpha (516011) ELISA (Cayman Chemical Company, Ann Arbor, MI, USA) kit as described in the manufacturer's instructions and presented as pg/mL.

Statistical Analysis

Normality and variance were analyzed using the One-Sample Kolmogorov-Smirnov test. Numeric data are presented as means and standard deviation, and categorical data as frequency and percentage. Oxidative stress marker levels were analyzed using the One-Way ANOVA test, and post-hoc comparisons were conducted using Tukey's honest significant difference test. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 20.0 program was used to complete all analyses. P values <0.05 were accepted as statistically significant in all analyses.

Results

All animals completed the experiment. No death or any complications occurred during the experimental exposure period. Slight fluctuations were detected in mean nitric oxide, prostaglandin E2, prostaglandin F2-alpha, superoxide dismutase, glutathione peroxidase, malondialdehyde, alginate dialdehyde, and xanthine oxidase levels; however, the differences were not significant (p>0.05, Table 1). The minimum levels of nitric oxide, prostaglandin E2, prostaglandin F2-alpha, superoxide dismutase, glutathione peroxidase, malondialdehyde, alginate dialdehyde, and xanthine oxidase were 0.75±0.07 micromol/L in S4, 501.63±149.29 pg/mL in S2, 0.11±0.01 U/mg in S4, 2.51±0.26 U/g in S1, 1.85±0.83 nmol/g in C, 55.38±29.09 nmol/g in S4, and 0.78±0.23 U/g in S5, respectively.

Discussion

The effect of reactive oxygen species appears to be a double-edged sword, they are used as signaling factors in physiologic conditions but also have additional roles in pathologic conditions including in the female reproductive system. There is continuous stability between oxidants and antioxidants. Superoxide dismutase, copper-zinc superoxide dismutase, and manganese superoxide dismutase are located in the

Table 1. Oxidative stress marker levels in rats exposed to sevoflurane

	C	S1	S2	S3	S4	S5	p
NO	1.07±0.30	0.77±0.06	0.96±0.09	0.98±0.49	0.75±0.07	0.91±0.17	0.344
PGE2	521.01±198.09	663.88±285.62	501.63±149.29	688.08±127.66	698.23±183.93	582.64±123.67	0.401
PGF2-alpha	138.09±56.77	111.12±13.99	89.25±29.07	110.09±40.69	136.64±77.55	145.89±27.94	0.376
SOD	0.16±0.04	0.12±0.01	0.14±0.01	0.15±0.07	0.11±0.01	0.14±0.02	0.337
GPX	3.29±1.64	2.51±0.26	3.03±0.59	4.24±1.96	2.58±0.24	3.20±0.32	0.187
MDA	1.85±0.83	2.14±1.00	2.04±0.40	2.44±1.07	2.30±0.23	2.07±0.91	0.892
ADA	71.90±15.91	77.74±18.95	101.79±72.92	76.82±10.27	55.38±29.09	74.23±32.62	0.534
XO	1.08±0.20	1.21±0.50	0.91±0.19	0.81±0.31	1.04±0.44	0.78±0.23	0.351

One-Way ANOVA test. Post-hoc comparisons were conducted by Tukey's HSD. No significant differences were detected in intra-group comparisons
NO: Nitric oxide; PGE2: Prostaglandin E2, PGF2-alpha: Prostaglandin F2-alpha, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, MDA: Malondialdehyde, ADA: Alginate dialdehyde; XO: Xanthine oxidase

granulosa and theca cells of developing follicles[17]. In addition, glutathione peroxidase activity can be seen in follicular fluid[18]. In contrast to the detrimental effects, oxidative stress may be one of the main factors that can manage ovarian germ and stromal cell physiology. Vascular changes and proteolytic cascades are the major factors regulating ovulation. The signaling between these two processes are established by vascular endothelial growth factor, reactive oxygen, reactive nitrogen species, and cytokines[17,18]. Ben-Shlomo et al.[18] showed that interleukin 1-alpha caused nitric oxide accumulation in rat ovaries, suggesting a possible interaction between cytokines and reactive nitric oxide species. Oxidative stress and cytokines are demonstrated as intercellular and intracellular messengers in rat ovarian tissues[19,20]. There is an evident balance between antioxidant enzymes and reactive oxygen species in the ovaries. Superoxide dismutase, one of the antioxidant enzymes, is intensely present in the theca interna cells of antral follicles. An experimental study revealed that luteal cells reduced the expression of estradiol and progesterone hormones after adding hydrogen peroxide (reactive oxygen species) to the cell culture environment[21]. The preovulatory follicle is heavily guarded against oxidative stress in which glutathione peroxidase is the major enzyme maintaining its lower hydroperoxide levels thus holds a crucial role in gametogenesis and fertilization[22]. In this context, the reactive oxygen scavenging system has an important role in all organ systems and the reproductive system. Environmental factors such as anesthetic agents may alter the fine balance of this regulatory mechanism.

The present study showed that acute exposure to sevoflurane by inhalation did not demonstrate any evident differences on oxidative stress markers of reproductive tissues in female rats. Various oxidative enzymes including copper-zinc superoxide dismutase, manganese superoxide dismutase, glutathione peroxidase, gamma-glutamyl-cysteine synthase, and catalase have an important role to protect the oocyte against the effects of oxidative damage during maturation and early preimplantation embryo development. Studies showed that codes for superoxide dismutase were ready to transcript in oocytes at all stages of maturation[23]. Oxidative damage may occur with several drugs and diseases, and shows a similar route associated with their development[24]. Various studies have been conducted on oxidative stress and inflammation, mainly focusing on isoflurane and sevoflurane[25]. Despite the limited data about the effects of sevoflurane on female ovary tissues, a study by Türkan et al.[26] on liver, kidney, brain, and lung of rats demonstrated that sevoflurane caused an increase only in the activity of anti-oxidative enzyme, malondialdehyde in lungs. Allaouchiche et al.[8] evaluated the impacts of sevoflurane and desflurane on lungs in mechanically-ventilated swine. Thereafter, they analyzed bronchoalveolar lavage fluid specimens and blood samples for levels of superoxide dismutase, glutathione peroxidase, and malondialdehyde. They found that sevoflurane led to an evident raise in malondialdehyde levels in both bronchoalveolar lavage fluid and plasma.

Free oxygen radicals have crucial roles in the normal immune defense system and metabolic activity. In contrast, the overproduction or disrupted elimination of these radicals may cause mild-to-severe cellular damage and DNA mutations by chemical modifications of cellular protein, carbohydrate, lipid, and nucleotides. Oxidative stress is described as the incline in enzymes of the anti-oxidant defense system[27]. Anesthetic agents may have a direct effect on the anti-oxidant system causing a decrease in the blood flow of the liver, thus leading to a relative increase in the magnitude of free oxygen radical production[28]. In contrast, with minor fluctuations detected in oxidative stress markers in the present study, sevoflurane showed no apparent disturbances in the anti-oxidative system of testicular tissue.

Study Limitation

Due to the financial aspects of experimental studies, we could not enhance the design of the study by including neither ovarian cell DNA nor immunohistochemical analysis. These kind of techniques could have provided detailed information about the effects of sevoflurane on the ovarian cell.

Conclusion

This is the first study to define the impacts of sevoflurane on the female rat reproductive system using oxidative system biomarkers. Our findings revealed that sevoflurane has no effect on the activity of anti-oxidant systems in the rat ovary. This result suggests that sevoflurane is a safe anesthetic agent in reproduction-aged females. Comprehensive studies are needed to confirm this outcome.

Ethics

Ethics Committe Approval: This study was approved by Animal Experiments Local Ethics Committee (2016-HADYEK-12).

Peer-review: External and internal peer reviewed.

Author Contributions

Concept: H.Y.D., S.D., Design: H.Y.D., İ.B., Data Collection or Processing: H.Y.D., İ.B., Analysis or Interpretation: H.Y.D., S.D., Literature Search: S.D., Writing: H.Y.D., S.D., İ.B.

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Could the female-to-male transgender population be donor candidates for uterus transplantation?

Kadından erkeğe cinsiyet değiştiren kişiler uterus transplantasyonu için donör olabilir mi?

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Abstract

Objective: To evaluate the eligibility of female-to-male (FtM) transgender people as donor candidates with regard to histologic, surgical, and social aspects.
Materials and Methods: In this prospective cohort study, 31 FtM transgender people underwent standard hysterectomy and bilateral salpingo-oophorectomy for gender reassignment upon their request. The pelvic viscera of the transgender people was intraoperatively observed and the histology of the removed uteri were evaluated for fertility capacity and procurement surgery. A questionnaire was administered to explore their attitude towards uterus donation.
Results: The mean ± standard deviation age was 28.5±5 years. The median duration of testosterone supplementation was 2.4 years; therefore, they all had irregular menstrual periods during this therapy. None had any previous abdominal surgery or additional morbidity. The mean uterine volume was 138±48 cm^3. No adenomyosis, endometriosis, polyps, adhesions or uterine anomalies were either observed or reported. Endometrial histology was reported as proliferative (58%), atrophic (29%), and secretory (13%) pattern. Of the 31 transgender people, 30 (96.7%) had a positive attitude; only one had no opinion at the beginning. After detailed information about the procedure was given, 26 (84%) still wanted to volunteer for donation, but 4 (12%) changed their opinion to negative (p=0.12, McNemar test).
Conclusion: The proposal of the FtM transgender population as uterus donor is a hypothetical model that has not been experienced before. Nevertheless, our experience revealed that the FtM transgender population would be good candidates socially, legally, and biologically.
Keywords: Uterus, transplantation, live donor, transgender people

Öz

Amaç: Kadından erkeğe cinsiyet değiştiren kişilerin uterus donörü olmaya uygunluklarını histolojik, cerrahi ve sosyal açıdan değerlendirmektir.
Gereç ve Yöntemler: Bu prospektif kohort çalışmada, 31 kişiye kadından erkeğe cinsiyet değişimi için laparoskopik histerektomi ve bilateral salpingo-ooferektomi uygulandı. Bu kişilerin pelvik viserası ve çıkarılan uterusların histolojileri üreme potansiyeli açısından değerlendirildi. Operasyon sonrası uterus donörü olmaya dair tutumlarına dair bir anket uygulandı.
Bulgular: Kişilerin ortalama ± standart sapma yaşı 28,5±5 yıldır. Ortalama testosteron desteği alma süresi 2,4 yıl iken destek süresince hastaların tümünün menstrüel periyotları düzensizdi. Herhangi bir kronik hastalık veya geçirilmiş abdominal cerrahileri yoktu. Çıkarılan uterus volümlerinin ortalaması 138±48 cm^3'tür. Herhangi bir adenomyozis, endometriozis, polip, adezyon veya uterus anomalisi gözlemlenmedi veya raporlanmadı. Endometrial histolojileri proliferatif (%58), atrofik (%29) ve sekretuar (%13) paternde rapor edildi. Otuz bir kişinin 30'u (%96,7) uterus donörü olma yönünde pozitif bir tutum bildirirken, bir kişi herhangi bir fikri olmadığını söyledi. Nakil için uterusun alınması operasyonu ile ilgili ayrıntılı bilgi verildikten sonra 26 (%84) kişi hala donasyon için gönüllü iken, 4 (%12) kişi kararını değiştirerek negatif tutum beyan etti (p=0,12, McNemar testi).
Sonuç: Kadından erkeğe cinsiyet değiştiren kişilerin uterus donörü olması daha önce denenmemiş hipotetik bir modeldir. Bu çalışma ile kadından erkeğe cinsiyet değiştiren kişilerin sosyal, hukuki ve biyolojik açıdan donör olmaya uygun adaylar olduğu düşünülebilir.
Anahtar Kelimeler: Uterus, transplantasyon, canlı donör, transgender

PRECIS: Female to male transgender population would be good for the candidates socially, legally and biologically.

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Introduction

Absolute uterine factor infertility (AUFI) is characterized by any condition that causes congenital/iatrogenic absence or non-function of uterus, such as severe intrauterine adhesions or multiple leiomyoma, which may destroy the complete architecture of the uterus[1-3]. Although congenital absence of the uterus Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome was estimated to be present in 1/4500 female births[4], AUFI affects one in every 500 women of reproductive age[5].

Adoption or gestational surrogacy are the currently available options to overcome childlessness in women with AUFI because an artificial uterus to support the embryo and carry the foetus till birth has not yet been invented. However, one or both of the options are forbidden or are not acceptable in several countries due to social, legal or religious reasons. For example, in Turkey surrogacy and in Egypt adoption are not legally approved. Uterus transplantation (UTx), despite it still being at a very experimental stage, is a reasonable option for fertility achievement. It differs from other organ transplantations with its temporary feature that has been kept until the recipient has delivered the desired number of children to limit the immunosuppression period. Therefore, it brings some important clinical and ethical considerations that need to be addressed. The ethical consideration has already been discussed by Farrell and Falcone[6]; therefore, it is beyond the scope of our paper. The clinical issues of this new procedure will be discussed in the context of donor candidates.

The first human UTx was carried out in 2000 in Saudi Arabia[7], with a uterus from a 46-year-old live donor. It was transplanted into a patient who had undergone emergency peripartum hysterectomy during her first childbirth. This attempt resulted in uterine prolapsus and necrosis followed by removal of the transplanted uterus 3 months after transplantation. The second UTx was reported by Ozkan et al.[8] from Turkey in 2013 in a recipient with MRKH syndrome who had undergone previous surgery for vaginal reconstruction. The uterus was procured from a brain-dead donor. Eighteen months after the UTx, the patient underwent two embryo transfer cycles[9]. The first cycle resulted in a biochemical pregnancy, and during the second attempt, an intrauterine gestational sac on sonography was confirmed as clinical pregnancy, but it was aborted. In 2014, Brännström et al.[10] initiated the first clinical trial of multiple transplantations, involving nine women who received uteri from live donors. After 6 months, seven uteri remained viable with regular menses and they reported the first successful UTx that resulted in a live birth with a weight of 1775 grams[11]. As of now, 11 human UTx have been reported and four healthy babies have been born from the aforementioned trial[12]. This report marks an important development to enable live births from women who lack a uterus.

In 2009, the International Federation of Obstetrics and Gynecology (FIGO) Committee[5] reported that it was unethical to remove a uterus for transplantation from young women who had not had the desired number of children. There seems to be an exception to the FIGO committee opinion. Female-to-male (FtM) transgender people, who voluntarily undergo hysterectomy, can be the most appropriate candidates for donation. The aim of this article was to scrutinize FtM transgender people as to whether they could serve as uterus donors, and to explore their attitude towards uterus donation (UD).

Materials and Methods

From March 2014 to November 2015, 31 FtM transgender people underwent hysterectomy and bilateral salpingo-oophorectomy upon their request after all the legal procedures regarding gender reassignment had been completed. Morphologic and histologic eligibility of the removed uteri were evaluated following surgery. The attitudes of the FtM transgender people towards UD were explored by conducting a survey composed of three choices: positive attitude, negative attitude or no opinion. A senior surgeon (MA) interviewed the transgender people and offered the survey before and after giving detailed information about standard hysterectomy and hysterectomy for procurement. The information about the procedures detailed the type of surgery (i.e. laparotomy/laparoscopy), duration of surgery and hospitalization, and potential complications. Written informed consent was obtained from all patients and the institutional review board approved the study. The McNemar test was used to compare the volunteers before and after giving detailed information about the procedure.

Results

In our cohort of 31 transgender people, the mean ± standard deviation age was 28.5±5 years. The patients were on testosterone therapy (Sustanon 250 mg/month, Schering-Plough) for at least two years. The median duration of testosterone supplementation therapy was 2.4 years; therefore, they all had an irregular menstrual history during this period. None had any previous abdominal surgery or additional morbidity. No adenomyosis, endometriosis, polyps, adhesions or uterine anomalies were observed or reported. Histologic examination revealed that the mean uterine volume was 138±48 cm^3. Two patients had intramural myomas with a maximum diameter of 2 cm. Endometrial histology was reported as proliferative (58%), atrophic (29%), and secretory (13%) pattern.

Of the 31-transgender people, 30 (96.7%) had positive attitudes; only one had no opinion at the beginning of the survey. After detailed information about the procedure was given, 26 (84%) still wanted to volunteer for donation, but 4 (12%) changed their opinion to negative (p=0.12, McNemar test).

Discussion

Procurement of a uterus from a live transgender person has some advantages; being young and healthy makes them ideal volunteers for donation. According to the evidence from studies on kidney transplantation, compared with recipients

of deceased-donor kidneys, recipients of living-donor kidneys wait less time for transplantation, have a lower risk of rejection, and have better allograft survival and longer life[13]. Moreover, the long-term graft survival of kidneys from live donors is superior to that of kidneys from deceased donors, possibly due to the fact that brain death induces organ injury and associated events. Living donors have to undergo extensive health and psychological assessment. Pre-donation procedures would be more rapid than any other living donors because transgender people have already undergone a two-year psychological and physical assessment for gender reassignment before the operation. Farrell and Falcone[14] commented, "Unlike other living-organ donors, who can expect continued organ system function (e.g., renal or hepatic), the uterus donor loses entirely her ability to have children." This may trigger some regrets about the donation. FtM transgender people are potential donor candidates who fully volunteer for donation and are more likely to have no regrets concerning this decision.

In the first clinical trial of UTx[10], seven of nine donors were close relatives of the recipients (their mothers or sisters) with a mean age of 53±7 years (Table 1). Unrelated living donors are becoming more common in other organ donations[15]. Although living donation in related donors have many advantages for overcoming donor-recipient incompatibility, advances in immunosuppressive therapy make the longevity and function of transplanted organs less dependent on the genetic donor-recipient relationship than in the past[13]. It seems that finding an unrelated donor will not always be easy. Either an unrelated living donor might need the uterus before the end of the reproductive age or the uterus might be useless as a healthy donor organ after the reproductive period because the uterus is a single organ. In the previously mentioned trial, the donors were selected among related postmenopausal women[10]. Previously, the effect of uterine aging on age-related decline in female infertility was studied and it was revealed that age-related reproductive failure was attributable to oocyte quality rather than the age of the uterus[16,17]. However, this evidence has come from older but normally menstruating women. Uterine aging may play a role in the reduction of endometrial receptivity, especially in elderly postmenopausal women. This issue needs to be further studied. On the other hand, these donors used combined oral contraceptives for 90 days before procurement to optimize uterine vasculature. This theoretical approach, which may increase the success of the transplantation, can place the donor in jeopardy of thromboembolism. All of these concerns are far from transgender people who are in their reproductive period. However, we can not obviate the fact that the uterus of transgender people has never harbored a pregnancy or has never been proven functional if it is transplanted. Besides, there is a lack of information as to whether androgen treatment affects the pregnancy potential of the uterus. The transgender patients stated that they had irregular menses under the testosterone supplementation, but when the therapy was suspended, their regular menstrual cycles resumed. It seems that the effect of androgen on the endometrium is transient[18]. According to our findings, the uteri of transgender people can be regarded as naïve sources with no morbidity. On this basis, transgender people would be considered as unrelated, readily available, young donors for the future.

Brännström et al.[11] developed a national awareness of this new parenthood option UTx in Sweden. In a recent report[19], the publics' attitude to UTx was examined and UTx was found to be more acceptable than surrogacy (80% vs. 47%, $p<0.001$). Surrogacy is not allowed in many countries, and also information on the surrogate mother and their families is scarce. In spite of its potential risk, UTx seems to be more reasonable because it provides intrauterine bonding between the mother and the child. In our study, most of the transgender people accepted the idea of being a uterus donor, but they had some doubts about the uterus procurement surgery. According to the Turkish Lesbian Gay Bisexual Trans-sexual Society records, 1500 FtM transgender individuals (1/25.333 female population) exist. We performed 31 gender reassignment operations over a period of 20 months. It has been estimated that 150 MRKH syndrome cases reach their reproductive ages each year. This rough estimation reveals that if all FtM transgender people agree to donate, this would supply a 10-year demand of UTx for the population with MRKH syndrome in Turkey.

UTx surgery entails isolation of the uterus with bilateral, long venous, and arterial vascular pedicles. The complexity of the surgery is mostly related to the extensive dissection of the pelvic sidewalls, which includes dissection of the ureters from their passages over the iliac vessel bifurcations distally to their inlets into the bladder, and dissection of the uterine veins and uterine

Table 1. Brief characteristics of previous uterus transplantations

Author, year	# cases	Recipient	Donor (age)	Uterus transplantation	Clinical pregnancy (#)	Live birth (#)
Fageeh et al.[7]	1	Peripartum hysterectomy	Live, unrelated (46)	Uterine prolapsus, necrosis	No	No
Ozkan et al.[8]	1	MRKH syndrome	Brain dead, unrelated (22)	Successful	Yes	No
Brännström et al.[11]	8 1	MRKH syndrome cervical cancer	Live, related (mean:53)	Successful	Yes (5)	Yes (4)

MRKH: Mayer-Rokitansky-Küster-Hauser

arteries from their firm attachments to the ureters. For a living donor, this brings some surgical complications including injury to major pelvic organs, life-threatening bleeding, and infection, amongst other problems. In Saudi Arabia[7], the donor's left ureter was damaged. Furthermore, in Sweden[10], one of nine donors presented with a utero-vaginal fistula. In addition to extensive dissection of the pelvis, it was reported that the donor surgery lasted 10-13 hours[10].

There is a need for a safe and easy alternative because the duration, complexity, and complications of the operation are unacceptably high for a donor. Transplantation surgery has to be more advanced by using the uterus with short pedicles containing the uterine artery and vein. If this hypothetical short pedicle technique could be achieved, the operations could be risk free and shorter for the donors. Another solution to ease the procedure is the use of other vascular anastomosis rather than uterine vessels. Kisu et al.[20] studied experimental surgical technique for UTx for a long time in Japan. Recently, they suggested that the ovarian vein could be used rather than the superficial or deep uterine vein, which were more difficult to dissect[20]. This proposal can make transplantation surgery less invasive and safe for donors. Long venous ovarian pedicles already exist because FtM transgender surgery encompasses bilateral salpingo-oophorectomy.

In all our case series, every transgender operation was performed via laparoscopy without any complications or conversion to laparotomy. Although laparoscopy has several advantages over laparotomy, by considering laparoscopic uterus procurement surgery, some questions have to be addressed with this specific issue:

1. It is well known that laparoscopy provides better abdominopelvic exploration; with laparoscope magnification, it enables fine dissection of vessels and pneumoperitoneum itself theoretically assists the development of pelvic avascular spaces. Besides these advantages, what are the limitations of laparoscopy in transplant surgery?
2. If an endoscopic approach would be preferred for the donor, could vascular pedicles be damaged during the extraction of the removed uterus through the vagina?
3. Would laparoscopic surgery be more acceptable for donors compared with laparotomy because of its better cosmetic results?

In the near future, UTx will be performed more commonly and the need for donors will be exceedingly debated.

Study Limitations

The results of the study are limited to the patients' characteristics and their attitudes toward UD. There is no information regarding the reasons motivating their decisions. In addition, the cohort is small, which could limit the validity of the study.

Conclusion

The proposal of the FtM transgender population as a uterus donor is a hypothetical model that has not been experienced before. Nevertheless, our experience has revealed that the FtM transgender population would be ideal candidates socially, legally and biologically.

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Ethics

Ethics Committee Approval: The study had been reviewed by the appropriate ethics committee and had been performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Informed Consent: Written informed consent was obtained from all patients and the institutional review board approved the study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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The efficacy of laparoscopic presacral neurectomy in dysmenorrhea: is it related to the amount of excised neural tissue?

Laparoskopik presakral nörektominin etkinliği çıkartılan nöral doku miktarı ile ilişkili midir?

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Abstract

Objective: To assess the correlation between the number of excised neural fibers and degree of pain relief following laparoscopic presacral neurectomy (LPSN).

Materials and Methods: In this before and after study, 20 patients with severe midline dysmenorrhea [Visual Analogue Scale (VAS) >80 mm] unresponsive to medical therapy were consecutively enrolled. All patients underwent LPSN. The superior hypogastric plexus was excised and sent for histologic confirmation. Two pathologists counted the number of neural fibers in the surgically removed tissue. VAS was used for pain assessment before and 2nd, 3rd, 6th, and 12th months after the operations.

Results: Out of the initial 20 patients undergoing LPSN, eight were excluded from the final analysis due to intraoperative diagnosis of endometriosis; therefore, the remaining 12 patients were evaluated. The pain scores significantly decreased at each follow-up visit compared with the preoperative period (p=0.002). The pathologists, who were blinded, reported the median (minimum-maximum) neural fiber count as 46 (20-85) and 47 (18-83). No significant correlation was demonstrated between the number of excised neural fibers and the amount of pain relief following LPSN.

Conclusion: LPSN is an effective surgical procedure to control primary dysmenorrhea. Our preliminary results revealed that the degree of pain relief in cases of severe midline dysmenorrhea was not related to the amount of excised neural tissue in LPSN.

Keywords: Dysmenorrhea, nerve fiber, presacral neurectomy, laparoscopy

Öz

Amaç: Laparoskopik presakral nörektomi (LPSN) sonrası ağrı miktarındaki azalma ile çıkarılan nöral doku miktarı arasındaki korelasyonun değerlendirilmesidir.

Gereç ve Yöntemler: Medikal tedaviye dirençli, şiddetli orta hat dismenoresi olan [Vizüel Analog Skoru; (VAS) >80 mm] ve LPSN yapılmaya karar verilen 20 ardışık hasta çalışmaya dahil edildi. Operasyonda eksize edilen süperior hipogastrik pleksus histolojik konfirmasyon için patolojiye gönderildi. Cerrahi olarak çıkarılan nöral fibriller iki patolog tarafından ayrı ayrı sayıldı. Operasyon öncesi ve operasyon sonrası 2., 3., 6. ve 12. aylarda hastaların ağrısı VAS ile değerlendirildi.

Bulgular: LPSN yapılan 20 hastanın 8'i intraoperatif endometriozis tanısı aldığı için analizden çıkarıldı, geriye kalan 12 hasta değerlendirildi. Ağrı skorlarının postoperatif dönemdeki her bir vizitte preoperatif dönem ile kıyaslandığında anlamlı oranda düştüğü izlendi (p=0,002). İki patolog tarafından rapor edilen medyan (minimum-maksimum) nöral fibril sayısı 46 (20-85) ve 47 (18-83) idi. Eksize edilen nöral fibril sayısı ile LPSN sonrasındaki ağrı azalması arasında anlamlı bir korelasyon bulunmadı.

Sonuç: LPSN primer dismoreyi tedavi etmede etkili bir cerrahi prosedürdür. Elde edilen ön sonuçlara göre ciddi orta hat dismorede LPSN ile eksize edilen nöral doku miktarı ile ağrı iyileşmesi arasında herhangi bir ilişki yoktur.

Anahtar Kelimeler: Dismenore, sinir lifi, presakral nörektomi, laparoskopi

PRECIS: The degree of pain relief after laparoscopic presacral neurectomy is not related to the amount of excised neural tissue.

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Introduction

Dysmenorrhea, painful menstrual cramps, is a very common gynecologic problem with a prevalence of 43-90% in women of reproductive age[1,2]. Dysmenorrhea is such a challenge that it interferes with the performance of daily activities, and may even lead to job or school absenteeism. Therefore, the main goal of treatment is to provide pain attenuation sufficient to sustain the woman's daily performance. Medical therapy is the primary treatment of dysmenorrhea, which includes oral contraceptive pills, systemic or local progestins, non-steroidal anti-inflammatory drugs, danazol and gonadotropin-releasing hormone analogues. Surgical therapies stay as the second line of treatment in refractory cases because medical therapies are associated with a failure rate of 20-25%[3,4]. Surgical intervention includes the interruption of a major group of cervical and uterine sensory nerve fibers, known as pelvic denervation. The presumed mechanism of presacral neurectomy (PSN) for pain relief of dysmenorrhea is primarily based on the anatomy of the sensory pathways from the pelvic viscera through the inferior and superior hypogastric plexus to the spinal column. Excision or incision of the superior hypogastric plexus, located in presacral area and so-called PSN, can disrupt many pain sensory pathways. The surgical technique of PSN was first described by Jaboulay[5] and Ruggi[6] as early as 1899. One hundred years after from the first description, Perez[7] reported the first case of laparoscopic PSN (LPSN).

LPSN is found to be an effective surgical procedure in most cases refractory to medical treatment[7-10]; however, some patients still can not benefit from this approach. This diverse clinical response to LPSN has not yet been elucidated. One explanation may be that other sensory pathways not included in the superior hypogastric plexus may be responsible for cases with partial or no pain attenuation. Another possible mechanism is that the amount of excised neural tissue during LPSN may not be sufficient enough to resolve dysmenorrhea. Factors that determine the degree of response to PSN have yet to be enlightened. We hypothesized that there was an association between the amount of excised neural tissue and the response to therapy. The aim of the present study was to assess the correlation between the number of excised neural fibers and the degree of postoperative pain relief following LPSN.

Materials and Methods

Between July 2013 and August 2015, patients with the sole symptom of dysmenorrhea (pelvic pain during menstrual periods) for more than 6 months were consecutively enrolled in the study. All patients underwent multidisciplinary evaluation by the urology, gastroenterology, physical therapy, and psychiatry departments to exclude other potential causes of pelvic pain. All patients were questioned and physically examined to determine the exact location of their pain. Patients with pelvic pain related to specifiable pathologic conditions such as psychiatric disorder, malignant or infectious disease, previous pelvic surgery, large uterine leiomyoma, diagnosis of visually or histologically confirmed endometriosis or adenomyosis, and/or patients with lateral pelvic pain were excluded.

The study population consisted of 20 patients, whose most prominent symptoms were severe midline dysmenorrhea [Visual Analogue Scale (VAS) >80 mm] that had been unresponsive to at least two alternate medical therapies for more than 6 months. All patients reported job or school absenteeism in each menstrual period.

Six of the 20 patients reported dyspareunia (painful sexual intercourse) and three had mild chromic pelvic pain (CPP) (pain that occurs below the umbilicus and lasts for at least six months) along with severe midline dysmenorrhea. Patients assessed for LPSN are summarized in a flow chart (Figure 1). Twenty patients underwent LPSN; however, during the laparoscopy, eight patients required additional endometriosis ablation or excision. To rule out the effect of additional procedures on postoperative pain levels, these patients were excluded from the final analysis.

Prior to the operation, all patients gave written informed consent for the details of surgical procedure including the risks, benefits, and recurrence rate of LPSN. A senior surgeon

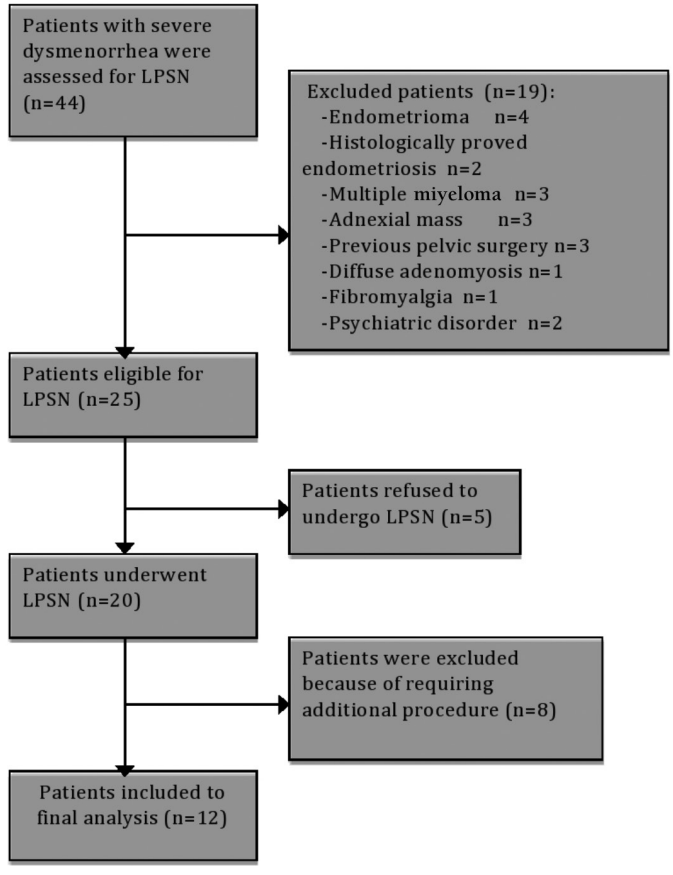

Figure 1. Flow chart of patients who were assessed for laparoscopic presacral neurectomy

LPSN: Laparoscopic presacral neurectomy

(M.A Massachusetts) performed all LPSN procedures with no intraoperative complications.

The severity of dysmenorrhea was assessed using a 100 mm VAS that ranged from "least possible pain" to "worst possible pain" at the time of hospital admission, and 2, 3, 6, and 12 months following the surgery. At admission, the women were requested to grade the most severe menstrual pain they experienced during the last 6 months using the VAS. The durations of the surgical procedures and hospital stay, blood loss, and intraoperative and postoperative complications were also recorded. All patients were followed up with clinical visits over 12 months to evaluate the degree of pain relief and possible complications. Zeynep Kamil Training and Research Hospital Review Board was approved our study. Written informed consent was provided from all patients to share and publish their medical records.

Surgical procedure

In the steep Trendelenburg position, the small intestines and sigmoid colon over the sacral promontory were pushed out laterally. After identifying the ureter on the right side, the peritoneum overlying the sacral promontory was incised transversally between the sigmoid mesentery on the left side and right ureter on the right side. The tissue on the sacral promontory, presumably the superior hypogastric plexus, was elevated as much to the lateral sides as possible, carefully dissected from the areolar tissue and then, excised approximately 1 cm above and below the L5-S1 disc level using a harmonic scalpel (Ethicon Endo Surgery, Cincinnati, Ohio). The removed plexus segment was sent for histologic evaluation.

Two pathologists (E.K. and H.Y.), who were blinded to the study interest, counted the neural fibers. The nerve specimens, after routine tissue processing, were cut into 3-µm-thick transverse, oblique, and longitudinal serial sections and deparaffinized in xylol for 10 minutes. They were dehydrated in a graded series of alcohol. The sections were kept in hematoxylin for 5-6 min. and washed with water for 5-10 min. They were then kept in eosin for 3-4 min and cleared in graded series of alcohol. After staining with hematoxylin-eosin, they were left to dry for a few minutes and placed in xylol. The tissues were passed through xylol three times for 10 min. Finally, they were taken out and mounted with a synthetic resin (entella). The sections were examined to determine the most appropriate sample, i.e., the section that included the greatest amount of neural fibers. To prevent repeated counting, the sections were mapped with a pencil (Figure 2).

Statistical Analysis

Statistical analyses were performed using the SPSS software version 20 (SPSS, Inc., Chicago, Illinois, USA). While investigating the associations between non-normally distributed or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. Friedman tests were used to compare the difference between the pre- and post-operative pain scores. In the post-hoc analysis of pairwise comparisons, the Wilcoxon test was performed. The agreement between the two pathologists for the neural fiber count was assessed using Lin's concordance correlation coefficient (ρ_c). A 5% type 1 error level was used to infer statistical significance.

Result

Out of the initial 20 patients undergoing LPSN, eight were excluded from the final analysis due to an intraoperative diagnosis of endometriosis. All of these eight patients had dyspareunia and/or CPP along with dysmenorrhea. The remaining 12 patients were evaluated for the final analysis. The median age was 29 years. All patients had midline dysmenorrhea with a median (minimum-maximum) duration of 11 (7-15) years. The median body mass index was 24.6 kg/m^2. The median (minimum-maximum) operation time for the LPSN was 32 (21-45) minutes. Blood loss was minimal. There were no minor or major vascular trauma, urinary or gastrointestinal complications related to LPSN. The patients were hospitalized for a maximum of 2 days postoperatively (median 1 day). There was no postoperative voiding dysfunction, constipation or other complications reported during the 12 months of follow-up.

Two pathologists reported the median (minimum-maximum) neural fiber count as 46 (20-85) and 47 (18-83). There was almost perfect agreement between the pathologists for the neural fiber counts [Lin's concordance coefficient (ρ_c)=0.99].

The median pain scores were 9.2, 3.3, 2.6, 1.7, and 1.6 before, and 2, 3, 6, and 12 months following the operation, respectively. Pain scores significantly decreased at each follow-up visit compared with the preoperative period (p=0.002). The reduction of pain scores remained stable from 6 to 12 months (p=0.08) (Figure 3).

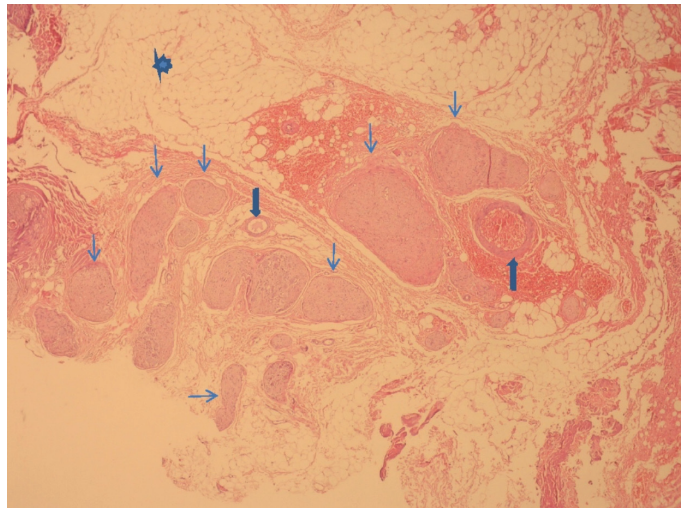

Figure 2. (Stained with hematoxylin and eosin, 40^x) Neural fibers surrounded by adipose tissue. Neural fibers are indicated by thin arrows; vessels are shown by thick arrows, and the star demonstrates adipose tissue

No statistically significant correlation was found between the neural fiber count and preoperative or postoperative pain scores (Table 1). After the procedure and throughout the follow-up period, all 12 patients reported no pain or mild pain without requiring pain medication.

Discussion

Up to 80% of reproductive aged women are affected by dysmenorrhea, in many cases causing sufficient pain that precludes social and occupational activities. Pain per se is a necessary defense mechanism of the body; however, chronic pain is a disturbing condition that needs to be treated. In persistent pain syndromes, people face distressing situations rather than biologic benefit. The presacral nerve, namely the superior hypogastric plexus, carries pain afferents from the cervix, the body of the uterus, and the proximal fallopian tube, but does not receive fibers from the ovaries and lateral pelvic structures. Therefore, PSN is traditionally performed for midline dysmenorrhea[11]. At this point, before deciding on surgical treatment, a detailed evaluation of patients to determine the location of pain has paramount importance in order to identify patients who would benefit from the procedure with the highest efficacy. In our study, we excluded subjects who reported lateral pain.

It has been shown that the success rate of PSN in the management of primary dysmenorrhea is 75 to 87%[12-14]. In the study by Jedrzejczak et al.[13], the efficacy of LPSN was evaluated in patients with and without endometriosis. Dysmenorrhea decreased at 3 months by 75% in those without endometriosis. Furthermore, these patients reported a significant decline in dyspareunia and pelvic pain unrelated to menses at 3 and 12 months after LPSN. In our cases, the pain scores significantly decreased over months. The median pain scores decreased sharply at the 2nd month, gradually from 2nd to 3rd month and then, remained stable. The patients with dyspareunia and chronic pelvic pain reported substantial pain relief that did not require any analgesic medication. Although the number of our participants is limited to 12 cases, we obtained 100% of efficacy in patients with severe dysmenorrhea and no identifiable pelvic pathology, when nerve bundles were excised.

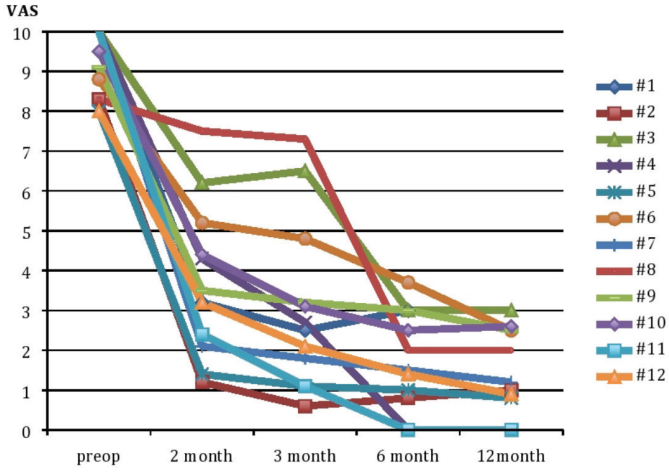

Figure 3. Visual Analogue Scale scores of 12 cases with dysmenorrhea before and 2nd, 3rd, 6th and 12th months after presacral neurectomy

VAS: Visual Analogue Scale

Previous studies reported that PSN might be ineffective in some cases. There is an on going debate as to whether efficacy could be related to the surgical method used. Some surgeons have asserted that a longer incision should be made on the hypogastric nerve plexus, whereas others claimed that the anatomic variation of nerve fibers at the area below the bifurcation of the aorta caused inconsistency in pain relief[15]. Furthermore, because the microscopic anatomy of a nerve plexus can not be differentiated during surgical exploration, surgeons only excise or transect the area where it is presumed to be the possible location of the superior hypogastric plexus. However, the architecture of the presacral area, which consists of fat, connective, vascular, and neural tissue, may mislead adequate excision of neural tissue. To ensure that the neural pathway has been included in the surgical area, histologic confirmation of the excised tissue is needed. On the other hand, the amount of removed neural tissue might be a determinant of surgical success. In our study, the number of neural fibers removed per specimen was counted by the pathologists. We detected that the neural fiber count differed from one patient to another and there was no correlation between the number of fibers removed per tissue and the amount of pain relief. This discrepancy can be explained by individual differences in the number of neural fibers or the complexity of pain perception. Moreover, the neural fiber intensity might not make any difference in pain transmission.

The method for PSN has been described in different approaches (laparotomy, laparoscopy, robotic) with different techniques (only transection or partial excision of nerve bundles) at different levels. Some preferred to excise a segment of 2-3 cm in diameter to prevent re-anastomosis of nerves, but others only transected without excision[16,17]. Chang et al.[15] compared LPSN with modified LPSN by transecting nerve bundles over different

Table 1. Correlation between the number of neural fibers evaluated by two pathologists and the pain scores at each interval

		Preoperative	At 2nd month	At 3rd month	At 6th month	At 12th month
1st Pathologist	Correlation coefficient	-0.25	-0.29	-0.17	-0.16	-0.23
	p value	0.42	0.35	0.6	0.6	0.46
2nd Pathologist	Correlation coefficient	-0.36	-0.6	-0.48	-0.36	-0.02
	p value	0.24	0.04	0.11	0.25	0.95

levels to evaluate long-term effectiveness in pain relief. In traditional LPSN, the presacral nerve bundle is transected over the presacral promontory, whereas in the modified method, it is transected over the aortic bifurcation. Although pain relief was sustained for five years in 90% of patients with both methods, recurrent pain was reported by 82% of the patients in LPSN and 43% in modified LPSN eight years after the operations (p=0.04). The authors commented that incomplete resection and re-growth of the nerve fibers might cause this difference. Presacral nerves spread out into a latticework at the level of the first sacral vertebra, where it divides into several branches going to the right and left sides of the pelvis[18]. These nerves lie beneath fatty areolar tissue contained in the anterior longitudinal ligament, the middle sacral artery, and the vein plexus, where it is too difficult to distinguish and dissect them completely. In addition, there are individual variations of pelvic anatomy and neurophysiology, intermingling of afferent fibers, intercommunication among nerve plexuses, and cross-talk[19]. Excision of the nerves may keep these factors under control and contribute to the long-term effectiveness of the LPSN. We observed a reduction of pain in all subjects supporting the theory that segmental resection might increase the effectiveness of the procedure.

Our study sheds light on the physiology of pain transmission through the superior hypogastric plexus. However, to our knowledge, no studies have identified any association between the efficacy of the procedure and the quantitative or qualitative parameters of excised neural tissue. Inter-observer variability assessment between the pathologists provides the safety of this experimental nerve counting method, which makes it reproducible for further studies.

Study Limitations

Our hypothesis was tested in a small number of cases. Further, the subtypes of the neural fibers were not differentiated.

Conclusion

Our preliminary results revealed that LPSN, if properly performed in selected cases, is an effective surgical procedure to control primary dysmenorrhea. According to the results of our study, the degree of pain relief in severe midline dysmenorrhea is not related to the amount of excised neural tissue in LPSN. The pathophysiology of pain transmission and perception, and the underlying mechanism of the effectiveness of neurectomy need to be further elucidated.

Ethics

Ethics Committee Approval: Zeynep Kamil Training and Research Hospital review board was approved our study.

Informed Consent: Written informed consent was provided from all patients to share and publish their medical records.

Peer-review: External and internal peer-reviewed.

Authorship contributions

Surgical and Medical Practices: M.A., A.B., M.C., Concept: M.A., A.B., Design: M.A., A.B., Data Collection or Processing: A.B., M.C., Analysis or Interpretation: M.A., A.B., M.C., E.K., H.Y., Literature Search: M.A., O.A., Writing: : M.A., A.B., M.C., E.K., H.Y., O.A.

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

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Sleep quality of endometrial cancer survivors and the effect of treatments

Endometrium kanseri olan hastalarda uyku kalitesi ve tedavinin etkisi

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Abstract

Objective: Sleep disorders affect 54.9% of gynaecologic cancer survivors. The effect of treatment methods on sleep quality is not clear. This study evaluated the sleep quality of survivors of endometrial cancer and compared the effects of different treatments on sleep quality.

Materials and Methods: Patients were categorised as surgery (group 1), surgery + brachytherapy (BRT) (group 2), surgery + external beam radiation therapy (EBRT) (group 3), and surgery + EBRT + BRT + chemotherapy (group 4). Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire. The PSQI was completed by the participants before surgery, 1, 3, and 6 months after each treatment was completed. The PSQI scores were compared between the different measurement times and different study groups.

Results: This study enrolled 114 patients with a mean age of 58.1±11 years. The number of participants in each group was 53 (46.5%), 14 (12.3%), 12 (10.5%), and 35 (30.7%), respectively. At baseline, 28 (24.6%) patients reported poor sleep quality. The mean PSQI score reached the maximum level at the second measurement and decreased slightly during follow-up and the change in the PSQI score was significant (p=0.001). Group 3 and group 4 had significantly higher scores from baseline (p<0.008). At time point 3, the differences between the groups were significant. At time point 4, the most prominent effect of treatment on sleep quality was observed in patients with combined chemo-radiotherapy when compared with the other study groups.

Conclusion: Most survivors of endometrial cancer are affected by poor sleep quality during their treatment. To improve these patients' quality of life, this disorder must be considered at each visit and tailored care plans should be developed to meet the women's needs. Further studies are needed to evaluate the long-term results of sleep quality on patients with endometrial cancer.

Keywords: Endometrial cancer, sleep disorders, sleep quality

Öz

Amaç: Uyku bozuklukları jinekolojik kanserli hastaların %54,9'unu etkilemektedir ve önemli bir sağlık sorunu oluşturmaktadır. Tedavi yöntemlerinin uyku kalitesine olan etkisi net değildir. Bu çalışma endometrium kanseri tedavisi alan hastalarda uyku kalitesinin değerlendirilmesi ve tedavi yöntemlerinin uyku kalitesine etkisini karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Hastalar farklı tedavi gruplarında değerlendirildi: (grup 1) cerrahi, (grup 2) cerrahi + brakiterapi (BRT), (grup 3) cerrahi + eksternal-ışın radyasyon tedavisi (EBRT) ve (grup 4) cerrahi + EBRT + BRT + kemoterapi. Uyku kalitesi Pittsburgh Uyku Kalitesi İndeksi (PSQI) anket formu kullanılarak değerlendirildi. Bu form katılımcılar tarafında ilk olarak tedavi öncesi doldurduldu. İkinci ölçüm her bir tedavi, yönteminin bitiminde 1 ay sonra ve sonrasında 3. ve 6. aylarda yapıldı. PSQI skorları farklı klinik gruplarda ve farklı ölçüm zamanlarında karşılaştırıldı

Bulgular: Bu çalışmaya 114 katılımcı dahil olmuştur. Çalışma grubunu ortalama yaşı 58,1±11'dir. Hastaların 53'ü (%46,5) sadece cerrahi, 14'ü (%12,3) cerrahi + BRT, 12'si (%10,5) cerrahi + BRT + eksternal radyoterapi ve 35'i (%30,7) hasta kemoradyoterapi tedavisi aldı. Başlangıçta, 28 (%24,6) hasta düşük uyku kalitesi bildirdi. Ortalama PSQI skoru ikinci ölçümde maksimuma ulaştı ve takipler boyunca azaldı. PSQI skorundaki değişim anlamlı bulundu (p=0,001). Üçüncü ölçümde gruplar arası karşılaştırmada anlamlı farklılık saptandı. Üçüncü ve dördüncü grupların ortalama skorları başlangıç skorlarında anlamlı yüksek saptandı (p<0,008). Dördüncü ölçümde tedavi yöntemleri içerisinde uyku kalitesine en belirgin etki kombine kemoradyoterapi alan hasta grubunda gözlemlendi.

Sonuç: Endometrium kanseri tedavisi alan hastaların birçoğu düşük uyku kalitesi sorunu yaşamaktadır. Hastaların yaşam kalitesini artırmak için bu durum her muayenede sorgulanmalı ve hastanın ihtiyaçlarının karşılayacak kişiye özel tedavi planları yapılmalıdır. Bu durumun uzun dönem sonuçlarının değerlendirilmesi için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Endometrium kanseri, uyku bozukluğu, uyku kalitesi

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PRECIS: We determined the effect of treatment on the sleep quality of endometrial cancer survivors by using special questionnaire.

Introduction

Sleep disorders affect an estimated 35% to 40% of adults[1]. These disorders affect 54.9% of survivors of gynaecologic cancer and constitute a prevalent health issue[2]. Sleep disorders have been shown to adversely affect the quality of life (QoL) of patients with ovarian, breast, and lung cancer[3-6]. Sleep disorders may arise at any stage of cancer as both cancer itself and therapeutic modalities may lead to sleep disturbance. Several mechanisms have been hypothesized for sleep disturbance in patients with cancer. Some inflammatory signals, tumour necrosis factor and C-reactive protein, as well as the effects of depression and distress are postulated as mechanisms of sleep disorders[7-10]. It was previously shown that radiotherapy affects the QoL of women with endometrial cancer (EC) probably due to radiotherapy-induced urologic symptoms in patients with cervical cancer[2,11]. Moreover, surgery seems to affect sleep quality mainly through surgical stress, pain, and medications. The role of chemotherapy remains controversial so far with few studies reporting inconsistent results. However, the impact of specific types of cancer on sleep still needs to be addressed because several important cofactors for sleep disorders such as age and sex may vary in particular cancer types.

EC is the second most common gynaecologic malignancy worldwide,[12] with an annual incidence of 300.000[13]. With improvements in diagnostic techniques and novel treatment modalities, most uterine cancers are diagnosed at an early stage, and patients have favourable prognoses[14-16]. So far, there are only robust data on the impact of EC on sleep quality. Therefore, the present study evaluated the sleep quality of EC survivors and compared the effects of different treatment modalities on sleep quality. In particular, we sought to determine if chemotherapy, radiotherapy or both as an adjuvant to surgery had any impact on sleep quality in patients with EC.

Materials and Methods

This study enrolled 114 patients with histologically proven EC who had completed treatment in the Selçuk University Faculty of Medicine Department of Gynaecological Oncology from 2012 to 2016. The study was approved by the Research Ethics Committee of Selçuk University (approval number: 2014/135). Written informed consent was obtained from all patients. All patients underwent a hysterectomy, bilateral salpingo-oophorectomy, and lymph node dissection. Debulking was performed as indicated for advanced-stage EC. All patients were staged according to the International Federation of Gynaecology and Obstetrics 2009 staging system for EC. For patients with stage Ia (<50% myometrial invasion) and grade 1 or 2 disease, adjuvant brachytherapy (BRT) was planned based on various risk factors, including patient age, lymphovascular space invasion, tumour size, and the presence of lower uterine (cervical glandular/stromal) infiltration. For women with grade 1 or 2 cancer and ≥50% myometrial invasion or grade 3 cancer and <50% myometrial invasion, vaginal BRT was performed. Patients with grade 3 cancer and ≥50% myometrial invasion or cervical stromal invasion were treated with external beam radiotherapy (EBRT). For women with high-risk early-stage disease or advanced disease, we administered EBRT ± BRT ± chemotherapy. Patients received concurrent paclitaxel (175 mg/m^2) and carboplatin (AUC=5), every 3 weeks for 6 cycles. Patients who received only BRT postoperatively were treated in five fractions at a dose of 5.5 Gy/fraction. Patients with EC who were treated with EBRT received 45.0 to 50.4 gray (Gy) with three-dimensional conformal radiotherapy using 18 mega volt photon beams. When EBRT and BRT were combined, we used the same EBRT dose with three fractions of BRT at a dose of 7 Gy/fraction. The inclusion criteria were histologically proven EC and age of >18 years. The exclusion criteria were recurrent EC, preoperative sleep disorder, the use of antipsychotic or anxiolytic drugs, and loss to follow-up. Demographic data, physical properties, medical comorbidities, and complications were recorded.

Measurement

Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), which comprises 19 self-rated items and incorporates 7 different components (subjective sleep quality, sleep latency, habitual sleep efficiency, night-time disturbances, sleep duration, use of sleep medications, and daytime dysfunction); the total score is the sum of the component scores. A total score of >5 indicates poor sleep quality. This questionnaire is an effective instrument for assessing the sleep quality of patients with cancer[17]. The PSQI was first completed by the participants before surgery (time 1). The time of the second questionnaire differed for each treatment method. Results were obtained after the final pathologic examination, and the patients were assigned to different treatment groups: surgery (group 1), surgery + BRT (group 2), surgery + EBRT (group 3), and surgery + EBRT + BRT + chemotherapy (group 4). The second measurement was made 1 month after each treatment was completed (time 2), and the test was repeated 3 (time 3) and 6 (time 4) months after treatment was completed. The PSQI scores were compared at different measurement times in the different study groups.

Statistical Analysis

Continuous variables were examined for normal distribution using the Kolmogorov-Smirnov test. Data are shown as the median (range) or number of cases and percentages where applicable. The mean values were compared between the groups using Student's t-test, and the Mann-Whitney U test was used to compare median values. Nominal data were analysed using Pearson's chi-square or Fisher's exact test where applicable.

Friedman's test was used to determine the statistical significance of differences among repeated clinical measurements. When the Friedman test yielded a significant p-value, Wilcoxon's 3 was used to identify which parameter was different. Bonferroni correction was applied to control for type 1 errors. Correlation analyses were performed to determine the correlations between sleep quality and variables. In all analyses, $p<0.05$ was taken to indicate statistical significance. The statistical analyses were performed with SPSS ver. 17.0 (SPSS, Chicago, IL).

Results

This study enrolled 114 patients with a mean age of 58.1±11 years. The final treatment was surgery for 53 (46.5%) patients, surgery + BRT for 14 (12.3%), surgery + EBRT for 12 (10.5%), and surgery + computed tomography + EBRT + BRT for 35 (30.7%). Ninety patients (78.9%) had type 1 EC and 24 (21.1%) had type 2 EC. The patients' characteristics are shown in Table 1.

Pittsburgh Sleep Quality Index scores

At baseline, 28 (24.6%) patients reported poor sleep quality (PSQI score of >5). There were no relationships between sleep quality and age, marital status, education status, economic status or menopausal status. A negative correlation was observed between body mass index and sleep quality (r=-0.189; p=0.4).

The mean PSQI score at time 1 was 4.59±2.80. The difference in the baseline PSQI score among the treatment groups was not significant (p=0.296). The score decreased slightly during follow-up. The change in the PSQI score was significant at each time point ($p<0.05$). Table 2 shows the mean PSQI score and rate of sleep disorders of the patients at the different measurement times.

At time 2, the PSQI score was 8.12±4.30, which was the maximum score reached. Poor sleep quality was reported by 79 (69.3%) patients. The differences between the study groups were significant ($p<0.05$). The difference between groups 1 and 2 was not significant. Group 3 had a higher PSQI score, but this was not significant. However, group 4 had a significantly higher score than the other groups ($p<0.008$) (Table 3). The rate of poor sleep quality was 60.4%, 64.3%, 66.7%, and 85.7% in groups 1 to 4, respectively. Patients in group 4 were more likely to have poor sleep quality than those undergoing other treatments [odds ratio (OR): 3.67; 95% confidence interval (CI): (1.28-10.49); p=0.011]. Subjective sleep quality, habitual sleep efficiency, and sleep duration were the most affected components of the PSQI in group 4.

At time 3, the mean PSQI score was 7.2±3.9. The differences between the treatment groups were significant (p=0.001). Group 4 had a significantly higher PSQI score than groups 1 and 2 ($p<0.008$). At time 3, the mean PSQI score in groups 1 and 2 was not significantly different from the baseline score, whereas groups 3 and 4 had higher scores ($p<0.008$) (Table 3). The rates of poor sleep quality were 22.6%, 35.6%, 66.7%, and 74.3% in groups 1 to 4, respectively. Patients in group 4 were more likely to have poor sleep quality than those receiving other treatments [OR: 6.24; 95% CI: (2.55-15.25); $p\leq0.001$].

At time 4, the mean PSQI score was 6.67±3.60. The differences between the treatment groups were significant (p=0.012). Group 4 had a significantly higher PSQI score than groups 1 and 2 ($p<0.008$), and group 4 had significantly poorer sleep quality (Table 3). Figure 1 shows the component scores and changes at each measurement time.

Table 1. Patient's characteristics

No. of patients	114
Age	58.1±11 (28-82)
Gravida	3.15±1.4
Parity	2.64±1.3
BMI (kg/m^2)	28.5±3.3
Hospital stay (day)	3.9±0.8
Marital status	**n (%)**
Single	3 (2.6)
Married	97 (85.1)
Divorced/widow	14 (12.3)
Disease characteristics	
Type 1 endometrial cancer	90 (78.9)
Type 2 endometrial cancer	24 (21.1)
Stage	
1A	48 (41.7)
1B	20 (17.5)
2	11 (9.6)
3A	6 (5.2)
3B	5 (4.3)
3C	17 (14.8)
4A	5 (4.3)
4B	2 (1.7)
Comorbidities	**n (%)**
Diabetes	25 (21.9)
Hypertension	39 (34.2)
Asthma	8 (9.1)
Valvular heart disease	4 (3)
Treatment	
Surgery	53 (46.5%)
Surgery + BRT	14 (12.3%)
Surgery + BRT + EBRT	12 (10.5%)
Surgery + EBRT + CT	35 (30.7%)

BRT: Brachytherapy, EBRT: External beam radiotherapy, CT: Chemotherapy, BMI: Body mass index

Discussion

To our knowledge, this is the first prospective study to evaluate sleep disorders and anxiety in Turkish women with EC. We followed up 114 patients for 6 months after the primary treatment was completed. The main findings of the present study are as follows: patients with EC have similar rates of sleep disturbance at diagnosis. Overall, sleep quality increases upon the completion of therapy, which then decreases gradually over time. Combined use of chemotherapy and radiotherapy has the greatest impact on sleep quality, but BRT had on adverse effect on sleep quality.

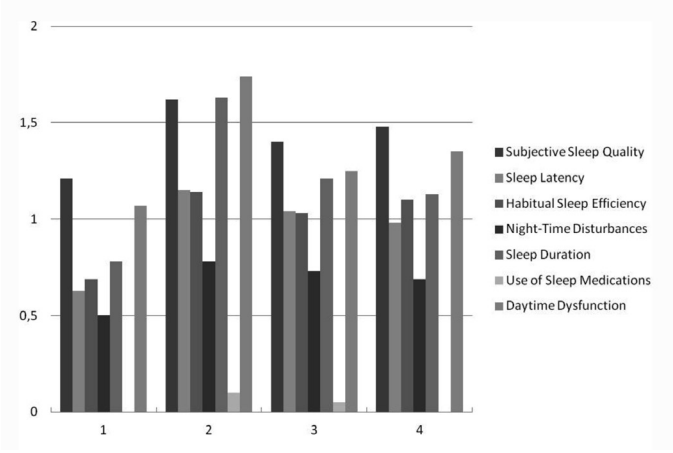

Figure 1. Component scores at each measurement time

It was suggested in several studies that release of several cytokines such as tumour necrosis factor and C-reactive protein, as well as the effects of depression and distress were the mechanisms of sleep disorders. There is also a relationship between sleep disorders and cancer-related fatigue[18], probably by desynchronisation of the circadian rhythm[19]. The relationship between noncancerous gynaecological conditions and poor sleep quality was observed in 33.7% of the participants with benign gynaecologic disease[20]. The importance of QoL on the prognosis of different cancer types was assessed in a global study and the authors reported that QoL predicted clinical outcomes well[21]. Before surgery, patients with ovarian cancer reported a high sleep disorder rate[22]. In ovarian cancer, abdominal discomfort might lead to poor sleep quality. Unlike previous studies, our data suggest that patients with EC had comparable sleep quality before treatment. In our study population, 24.6% of women had poor sleep quality before treatment, similar to that of the healthy population. We believe this difference may

Table 2. Comparison of Pittsburgh Sleep Quality Index scores at different measurement times

	Time 1	Time 2	Time 3	Time 4	p
Whole patients					
PSQI score¥	4.59±2.80	8.12±4.30[a]	7.20±3.90[a,b]	6.67±3.60[a,b,c]	0.001*
Poor sleep quality, n (%)β	28 (24.6)	79 (69.3)	74 (64.9)	68 (59.6)	<0.001*
Group 1¥					
PSQI score¥	4.05±2.17	6.86±0.40[a]	5.79±3.80[b]	5.90±3.40[b]	<0.001*
Group 2¥					
PSQI score¥	4.50±2.70	6.28±2.80[a]	4.92±1.50	5.42±3.10[b]	0.001*
Group 3¥					
PSQI score¥	4.16±2.60	6.58±2.70[a]	6.62±1.40a	5.25± 3.10[b,c]	0.004*
Group 4¥					
PSQI score¥	5.62±2.60	11.11±4.90[a]	9.11±4.70[a,b]	8.42±3.60[a,b,c]	<0.001*

¥Friedman test, βChi-square test, PSQI: Pittsburgh Sleep Quality Index, *Statistically significant p-value
Significant differences from times [a]1, [b]2, and [c]3 (all p<0.008) after Bonferroni correction
Time 1: Before surgery, Time 2: 1 month after treatment, Time 3: 3 months after treatment, Time 4: 6 months after treatment

Table 3. Comparison of Pittsburgh Sleep Quality Index scores of different treatment groups

	Group 1	Group 2	Group 3	Group 4	p
Time 1	4.05±2.17	4.50±2.70	4.16±2.60	5.62±2.60	0.296
Time 2	6.86±3.40	6.28±2.80	7.02±2.70	11.11±4.90[a,b,c]	≤0.001*
Time 3	5.79±3.80	4.92±1.50	5.58±1.40	9.11±4.70a,[b]	0.001*
Time 4	5.90±3.40	5.42±3.10	6.41±3.30	8.42±3.60[a,b]	0.012*

*Statistically significant p-value
Significant differences from times [a]1, [b]2, and [c]3 (all p<0.008) after Bonferroni correction

be related to the nature of EC which, unlike ovarian cancer, is generally localised at diagnosis and lacks abdominal symptoms. Many studies have suggested an association between surgery and poor sleep quality[23,24]. Sleep fragmentation and reduced sleep time are the primary symptoms observed after surgery. Surgical stress, pain, and medications are suggested causes of poor sleep quality[23,24]. In our study cohort, the mean PSQI score increased significantly compared with the baseline scores and 79 (69.3%) patients had poor sleep quality postoperatively. Subjective sleep quality, sleep disturbances, and daytime dysfunction are the most affected components of the PSQI by surgery. Our data are in keeping with these observations because our cohort had the highest rate of sleep disturbance immediately after surgery (time 2).

Radiotherapy affects the QoL of women with EC, as shown in many studies[2,11]. There is an association between poor sleep quality and radiotherapy in patients with cervical cancer[25]. The potential mechanisms of the poor sleep quality after radiotherapy could be that the radiotherapy induces urologic symptoms[2]. Assessment of bladder symptoms in cervical and EC survivors showed that radiotherapy for EC was associated with nocturia, and BRT was associated with the severity of the nocturia[26]. In the present cohort, patients receiving EBRT alone as an adjuvant therapy had PSQI scores similar to patients receiving BRT or no adjuvant therapy. Daytime dysfunction, sleep latency, and subjective sleep quality were the components most affected after EBRT. Another study showed the impact of radiotherapy on the QoL of patients with cancer and concluded that the negative effect of radiotherapy was temporary and improved 1 month after treatment was completed[27]. In our study cohort, the PSQI scores were improved slightly at 3 months, but this was not significant. Six months after treatment, the improvement was significant. This result was similar to previous studies. The effect of radiotherapy on sleep quality continues for at least 3 months after radiotherapy is completed.

The effect of chemotherapy on sleep quality is controversial. In breast, prostate, and ovarian cancer, high rates of sleep disorders before treatment and improvement after treatment have been reported[4,28]. Poor sleep quality during chemotherapy for rectal cancer has also been reported[29]. Palesh et al.[30] assessed the association between cancer and sleep quality and concluded that the rate of insomnia was three times higher in patients receiving chemotherapy. Patients receiving combined chemo-radiotherapy are more likely to develop sleep disorders. In our study, The PSQI scores were significantly higher in the chemo-radiotherapy group at all follow-up times. In the chemo-radiotherapy group, subjective sleep quality, habitual sleep efficiency, and sleep duration were the most affected components of the PSQI. We believe the adverse effect profile of the present chemotherapy regimen such as neurotoxicity, emesis, and nephrotoxicity might account for decreased sleep quality in patients who received chemotherapy.

Study Limitation

Our study has some limitations that deserve mention. First, it was conducted at a single centre and the patient group was relatively small and heterogeneous. We did not evaluate symptoms that might affect sleep quality. Moreover, treatments could not be randomly assigned and sleep quality was assessed by only subjective sleep measures. However, this study was a prospective study and allowed for comparison different treatment methods.

Conclusion

Most survivors of EC are affected by poor sleep quality during their treatment. The effects of surgery and BRT on sleep quality appear to be short-term effects. Combined treatment with radiotherapy and chemotherapy seemed to have more severe, long-term effects on sleep quality. This conclusion is based on changes in sleep quality scores at each timeline during treatment. However, we were unable to identify whether the higher rate of sleep disturbance was due to more advanced disease or the therapy itself. Further studies are needed to evaluate the long-term results of sleep quality on patients with EC.

Ethics

Ethics Committee Approval: The study was approved by the Research Ethics Committee of Selçuk University (approval number: 2014/135).

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

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ç.Ç., T.T.İ., A.G., Concept: Ç.Ç., Design: T.T.İ., M.G.U., G.Y., Data Collection or Processing: T.T.İ., A.G., Analysis or Interpretation: T.S.İ., Literature Search: T.T.İ., T.S.İ., Writing: T.T.İ., T.S.İ., A.G.

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

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Case Report / Olgu Sunumu

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Molar pregnancy in cesarean section scar: A case report

Sezaryen skarında molar gebelik: Olgu sunumu

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Abstract

Cesarean scar ectopic pregnancies and molar pregnancies are two very rare obstetric pathologies. In both cases, serious morbidities are involved that require careful management. The coexistence of the two clinical conditions is far less common and there are a limited number of cases in the literature. In this case report, a 34-year-old patient with previous cesarean section was diagnosed as having a molar pregnancy in a cesarean scar through ultrasonography. The patient was asymptomatic at that time. Ultrasonography revealed a protruding mass at the cesarean section and her human chorionic gonadotropin level was measured as 59.705 mIU/mL. Due to the risk of severe bleeding, cesarean section scar excision and revision were performed via laparotomy after counselling the patient. Removal of all trophoblastic tissue was observed as a result of the frozen pathology and the operation was terminated. After the definite pathology result came as a complete molar pregnancy, the patient was followed up according to molar pregnancy follow-up protocols and cured completely. Despite the alternative treatment options (methotrexate application, curettage, uterine artery embolization) in such patients, the decision for surgery was made after counselling the patient. In this very rare clinical condition, patients should be closely monitored and the appropriate treatment option should be applied as soon as possible, taking into consideration the bleeding risks of both pathologies.

Keywords: Cesarean scar pregnancy, molar pregnancy, management

Öz

Sezaryen skar ektopik gebelikleri ve molar gebelikler çok nadir görülen iki ayrı obstetrik patolojidir. Her iki durumda da ciddi morbiditeler söz konusudur ve dikkatli bir yönetim gerektirmektedir. İki klinik durumun bir arada görülmesi ise çok daha nadir durumdur ve literatürde sınırlı sayıda olgu vardır. Bu olgu sunumunda 34 yaşında geçirilmiş sezaryen öyküsü olan hasta asemptomatik olarak başvurmaktadır ve yapılan ultrasonografi sonucunda sezaryen skarına yerleşmiş molar gebelik ön tanısı oluşmuştur. Ultrasonografide skar üzerinde yerleşen protrude bir kitle izlenmiş ve insan koryonik gonadotropin düzeyi 59,705 mIU/mL olarak tespit edilmiştir. Ciddi kanama riski nedeniyle hastaya operasyon seçeneği sunularak laparotomi ile sezaryen skar eksizyonu ve revizyonu yapılmıştır. Frozen patoloji sonucunda tüm trofoblastik dokunun çıkarıldığı gözlemlenmiş ve operasyon sonlandırılmıştır. Kesin patoloji sonucunun da komplet mol gebelik olarak gelmesi sonrası hasta mol gebelik takip protokollerine uygun olarak takip edilmiş ve kür sağlanmıştır. Bu tür hastalarda alternatif tedavi seçenekleri (metotreksat uygulanması, küretaj, uterin arter embolizasyonu) olmasına karşın hasta ile tüm riskler konuşularak cerrahi kararı verilmiştir. Çok nadir olarak rastlanan bu klinik durumda her iki patolojinin kanama riskleri de göz önünde bulundurularak hastalar yakından takip edilmeli ve uygun tedavi seçeneği en kısa sürede uygulanmalıdır.

Anahtar Kelimeler: Sezaryen skar gebelik, molar gebelik, yönetim

Introduction

Molar pregnancy is mostly seen in the uterine cavity with a frequency of 1/10000. Ectopic pregnancy (EP) is seen more frequently with an overall frequency of 20/1000, and mostly located in the salpinx. Despite the rarity and different clinical spectrum of the diseases, it may be seen together as a very rare entity, which has been reported with a incidence of one per million pregnancies[(1)].

Here in, we report a case of molar pregnancy in a cesarean section scar that was diagnosed and managed with surgery at our clinic.

Case Report

A woman aged 34 years with a history of one vaginal delivery and one cesarean delivery was hospitalized in our clinic with a diagnosis of cesarean scar EP. The patient had no symptoms at that week of pregnancy. The gestation was calculated as 5 weeks

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according to the last menstrual period. Transvaginal ultrasound revealed a material in the cesarean scar, which reached the uterine serosa and protruded from anterior uterine wall with dimensions of 28x24 mm. The human chorionic gonadotropin (hCG) level was measured as 59.705 mIU/mL. The patient was suspected as having a molar pregnancy as an initial diagnosis and the possible medical and surgical options were presented to the patient. A surgical approach was chosen as the primary approach and the patient underwent surgery after we acquired informed consent. During the operation, 50x40 mm of pregnancy material was observed in the old cesarean section. After dissecting the bladder from the peritoneum, EP material was seen as a whole reaching the right corner of the old scar (Figure 1).

The old cesarean scar was incised to reveal the extensions of the molar tissue. Whole trophoblastic tissue was excised through a wedge resection reaching the normal tissue both in the upper and lower segments. The incision was repaired using a double-layer suture after ensuring that no trophoblastic tissue remained. The material was evaluated via frozen section because of the suspicion of molar pregnancy. The result was reported as complete molar pregnancy with negative surgical borders (Figure 2).

The hCG level was 7049 mIU/mL on the second postoperative day. The patient was discharged with hCG follow-up according to molar pregnancy follow-up protocols. The hCG level was 3.2 mIU/mL at the first postoperative month and negative at the first 6-month follow-up. Informed consent was acquired from the patient to publish this case report.

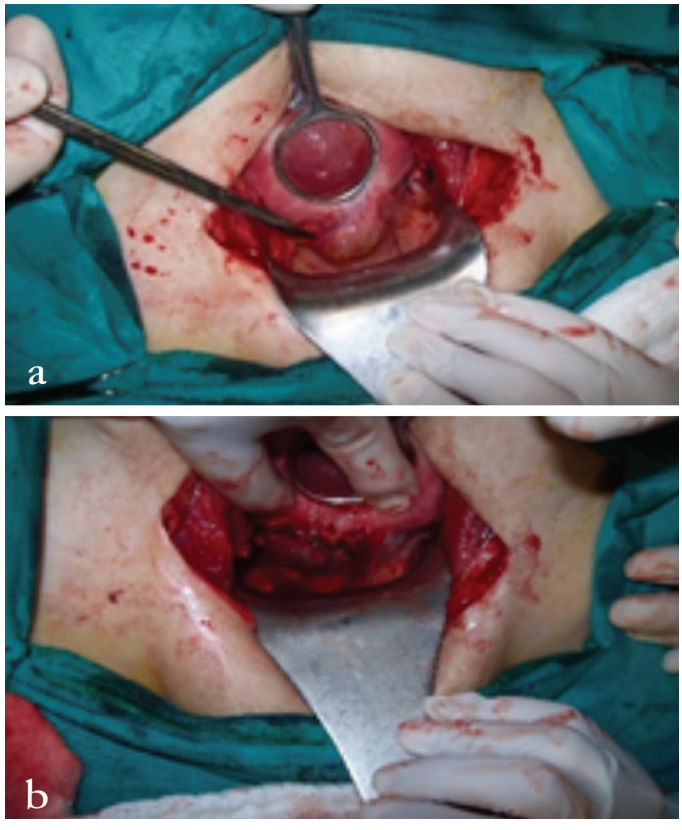

Figure 1. (a) Protruted mass at the cesarean scar (b) Mass reaching the right corner of the cesarean section scar

Discussion

EP is a complication of pregnancy in which the embryo attaches to sites beyond the endometrium, mostly the tuba uterina. Patients mostly admit with vaginal bleeding or severe abdominal pain; EP may also be diagnosed in patients who have no symptoms. Beyond some rare forms of EP, cesarean section scar pregnancy is among the rarest form of EP with an incidence of 1:1800 to 1:2216[2]. Cesarean scar molar pregnancy is even more rare due to the rarity of the coincidence of these 2 rare conditions. According to a literature search, 3 cases of cesarean scar molar pregnancy have been reported. Molar pregnancies with ectopic implantation were mostly seen in the fallopian tubes according to case series of molar EPs[3].

The first case was reported by Wu et al.[4] in 2006. The patient was admitted with vaginal bleeding, which was diagnosed as partial molar pregnancy, and a suction curettage was performed. After on going bleeding after one week, the patient was evaluated again and residual molar tissue was observed in the cesarean scar tissue. Secondary suction curettage was performed with ultrasonographic guidance and treatment was completed.

The second case was reported by Michener and Dickinson[5] in a case series in 2009. One of 13 cases of cesarean scar EP was reported as a molar pregnancy. After administration of methotrexate systematically and into the gestational sac, the patient was followed up. At the 10th month, the patient was admitted with vaginal bleeding requiring hysterectomy. After pathologic confirmation of molar tissue in the hysterectomy specimen, the patient was evaluated as having molar pregnancy on the cesarean section scar.

The third case was presented by Ko et al.[6] in 2012. The patient was admitted with suspicion of retained tissue after pregnancy termination at another clinic for a 7-week

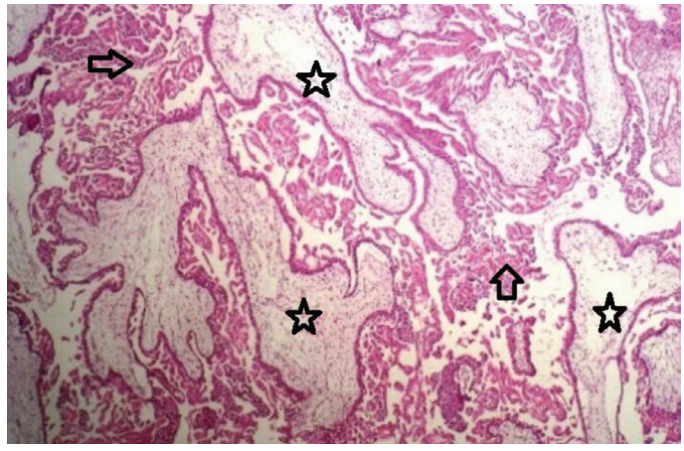

Figure 2. Hydropic villi surrounded completely by proliferative trophoblasts. Arrows indicating proliferating trophoblasts and (*) indicating hydropic villi

pregnancy. Transvaginal ultrasound revealed a suspected molar pregnancy in the cesarean section scar and for histopathologic confirmation, suction curettage was performed. Uterine artery embolization was performed for definitive treatment.

Our patient was admitted to our clinic for routine examination and had no symptoms. Cesarean scar EP was the initial diagnosis. Jurkovic et al.[7] defined the diagnostic criteria for cesarean scar EP as follows: a) Empty uterine cavity and cervix; b) Thinning of the myometrial layer between the bladder and gestational sac; c) Determination of peritrophoblastic perfusion around the gestational sac using Doppler sonography; d) Non-changing position of the gestational sac after gentle pressure from a transvaginal ultrasound probe[7]. Our case was consistent with all these findings and cesarean scar EP was the initial diagnosis for this patient. The reason for our suspicion of molar pregnancy in this case was the absence of a gestational sac, existence of extending tissue beyond the uterus, the incompatible value of hCG with the gestational week, which was calculated according to the last menstrual period.

In the treatment of cesarean scar pregnancies, systemic or direct methotrexate admission, wedge resection by laparotomy or laparoscopy, dilatation and curettage, curettage by hysteroscopy, and uterine artery embolization or a combination of these modalities are used[8]. Ultrasound-guided suction curettage is accepted as a reliable first-line treatment[9]. Suction curettage alone or in combination with other medical interventions has been evaluated as successful according to complications and success rates in case series[10]. Cesarean scar pregnancies may also result a high burden of maternal morbidities including severe hemorrhage, early uterine rupture, and hysterectomy with expectant management[11]. These pregnancies should be diagnosed carefully to manage patients with minimal morbidities. hCG levels, myometrial thickness, and gestational week must be evaluated to determine the proper approach to minimize morbidities[12].

The decision for laparotomy was made with the suspicion of gestational trophoblastic disease and the high risk of bleeding and perforation. Local methotrexate administration has been reported as a more risky treatment modality for cesarean scar EPs[5].

Cesarean scar pregnancies are usually identified with ultrasonography and diagnosis maybe delayed. Cesarean section molar pregnancy is a challenging diagnosis and hard to diagnose correctly preoperatively, mostly due to its rarity. Pregnancy localizations should be determined early in pregnancies of patients with past uterine scar or cesarean history and cesarean scar pregnancy should be among our differential diagnoses in such risky pregnancies. The factors mentioned in our case and other cases must be kept in mind so as to acquire the correct diagnosis in these rare cases. Early diagnosis and treatment may be life-saving in such rare cases.

Ethics

Informed Consent: Informed consent was acquired from the patient to publish this case report.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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

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Case Report / Olgu Sunumu

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Uterine rupture in pregnancy subsequent to hysteroscopic surgery: A case series

Histeroskopi sonrası gebelikte uterin rüptür: Olgu serisi

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Abstract

Uterine rupture during pregnancy is associated with high mortality and morbidity rates in both the fetus and the mother. Hysteroscopic surgeries such as myomectomy and septum resection are known risk factors for uterine rupture in pregnancy following the operation. We present four infertile patients who were admitted to Kocaeli Medical Park Hospital between February 2014 and November 2016. Three of the patients underwent hysteroscopic septum resection without complication and one had hysteroscopic myomectomy and a 7-8 mm sized rupture was detected. All of the patients became pregnant in less than a year after the operations. The first three patients had uterine rupture at 22nd, 38th, and 10th week, which is the earliest rupture in the literature. The last patient had an uneventful pregnancy and the rupture was observed during cesarean section. A short interval between hysteroscopy and pregnancy may increase the risk of rupture. It may be possible to become pregnant despite rupture and not have any problems during the entire pregnancy.
Keywords: Hysteroscopy, uterine rupture, pregnancy

Öz

Gebelik sırasında uterin rüptürü hem anne hem fetüs için yüksek mortalite ve morbidite ile ilişkilidir. Miyomektomi ve septum rezeksiyonu gibi histeroskopik operasyonlar, bu operasyonu takip eden gebeliklerde uterin rüptür için bilinen risk faktörleridir. Bu çalışmada Şubat 2014 ve Kasım 2016 arasında Kocaeli Medical Park Hastanesi'ne başvuran dört infertil hasta sunulmaktadır. Üç hastanın histeroskopik septum rezeksiyonu komplikasyonsuz olarak tamamlanmış olup, dördüncü hastada histeroskopik miyomektomi sırasında 7-8 mm boyutunda bir rüptür tespit edilmiştir. Hastaların hepsi histeroskopiden bir yıldan daha az süre sonra gebe kalmışlardır. Uterin rüptürler ilk üç hastada 22., 38. ve 10. haftalarda gerçekleşirken, sonuncu hasta sorunsuz bir gebelik geçirmiş ve rüptür sezaryen sırasında fark edilmiştir. Histeroskopi ve gebelik arasında geçen sürenin kısalığı uterin rüptür riskini artırıyor olabilir. Uterusta bulunan bir rüptüre rağmen gebe kalmak ve tüm gebeliği sorunsuz geçirmek mümkün olabilir.
Anahtar Kelimeler: Histeroskopi, uterin rüptür, gebelik

Introduction

Hysteroscopy is a routinely applied procedure for cervical canal and uterine cavity visualization. It is preferred in diagnosis, sample taking, and also in intrauterine surgeries. In septum resection, synechiolysis, myomectomy of submucosal myomas, and polypectomy, hysteroscopy is accepted as the standard treatment. Although diagnostic hysteroscopy is significantly safe compared with surgical hysteroscopy, there is a rate of 0.95% complications in surgical applications[(1)]. The complications can be listed in the order of frequency as perforation (instrumental, hysteroscopic) and fluid overload. The rate of complications in different procedures of hysteroscopy differ. Although adhesiolysis has significantly higher incidence of complications, it is followed by endometrial resection, myomectomy, and polypectomy, which are not significantly different from each other[(1,2)].

Uterine rupture is both intra-operative and one of the late complications of hysteroscopy. Although it is a rare complication,

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it can be catastrophic if not managed properly and may result in fetal and maternal death. Uterine perforation and metroplasty (both abdominal and hysteroscopic) are risk factors of uterine rupture in subsequent pregnancies[3,4]. Physiologic distention of the uterus during pregnancy may increase the risk of rupture in scarred uteruses.

The purpose of this study was to present four cases of uterine rupture during pregnancy following a hysteroscopic surgery in 4 infertile patients who were admitted to Kocaeli Medical Park Hospital between February 2014 and November 2016. Approval was obtained from the local ethics committee to perform the study. Written informed consent was obtained from all participants.

Case Reports

Case 1

A 24-year-old nulligravida, nulliparous woman presented with four years of primary infertility. The investigations of hysterosalpingograhy and 3D ultrasonography (USG) showed uterine septum and later the septum was resected hysteroscopically. During the 6 months of follow-up, the patient could not become pregnant. Despite gonadotropin application for two cycles and insemination because of astenospermia, the infertility of the patient continued. The patient was then treated with short-protocol *in vitro* fertilization (IVF) for one cycle. She had an uneventful pregnancy until she was admitted to hospital with abdominal pain at the 38th week. Despite reactive external fetal tocography without uterine contractions, cesarean section was performed because the patient's pain persisted. A rupture with disruption of all layers measuring 4 cm was observed in the uterine fundus.

There was no accompanying intra-abdominal bleeding, fetus or amniotic sac protrusion. By lower segment transverse incision, a 3150 g male infant with Apgar score of 9-10 was delivered by its foot. The placenta was completely exteriorized from the uterus. Uterine rupture was repaired through a double-layer closure with 1.0 absorbable sutures. The mother and child were discharged two days later without any complications or problems and antibiotic therapy was given. No other problems were observed during the one-month follow-up of the patient.

Case 2

A 39-year-old woman, gravida 6, abortus 5, with no living babies, underwent hysteroscopic surgery because of bicornuate uterus and complete septum. After her septum was resected hysteroscopically, in the laparoscopy a 1.5 cm sized indentation on the fundus was observed, and both tubes were open. No perforation was detected. Six months later, she became pregnant with twins through Klomen (Clomid) induction. The patient had no problems during her pregnancy until she presented to the clinic at the 10th week with periumbilical pain and bleeding. She underwent an emergency operation, and uterine rupture was detected on the fundus and closed. Six months later, the size of the rupture was measured as 5 mm using USG. She was followed up for three months and could not become pregnant with clomiphene citrate. After IVF treatment for one cycle, she had no results again. Six months after undergoing the Strassman metroplasty, controlled ovarian hyperstimulation IVF was started; follicle growth was not detected and the treatment was cancelled. She was followed up for natural cycles. In the 4th month, human chorionic gonadotropin was applied for one follicle, and she became pregnant. Her pregnancy was uneventful and a 3200 g infant was delivered by elective cesarean section at the 38th week.

Case 3

A 27-year-old woman, gravida 3 (1 ectopic, 1 chemical), parity 1, with no living babies, who had uterine septum with thickness of 2 cm, underwent hysteroscopic septum resection. No complications or uterine rupture were observed in the hysteroscopy. During the 6-month follow-up she could not become pregnant. IVF treatment with 3 embryo transfers was then applied. Her singleton pregnancy was uneventful until the 22nd week, when she was admitted to hospital on foot with persistent right subcostal pain beginning in the night and vaginal spotting. On USG, amniotic membranes protruding from posterior side of the uterus were observed. She was underwent surgery immediately. In the operation, the uterine rupture measured 3 cm and was repaired using double-layer closure with 1.0 absorbable sutures. She was followed up for one year and no extra problems were observed.

Case 4

A 32-year-old nulliparous and nulligravida woman admitted to our clinic after reporting not being able to become pregnant during 2 years of marriage (December, 2014). She had regular but excessive bleeding. A type 3 23x18 mm submucous myoma was detected in the fundal region using transvaginal USG. During the hysteroscopic myomectomy, a 7-8 mm sized rupture near the fundus was observed (Figure 1). After the bleeding was controlled, the operation was ended. The patient was discharged a day after the operation after the vital signs were checked and an abdominal examination was performed.

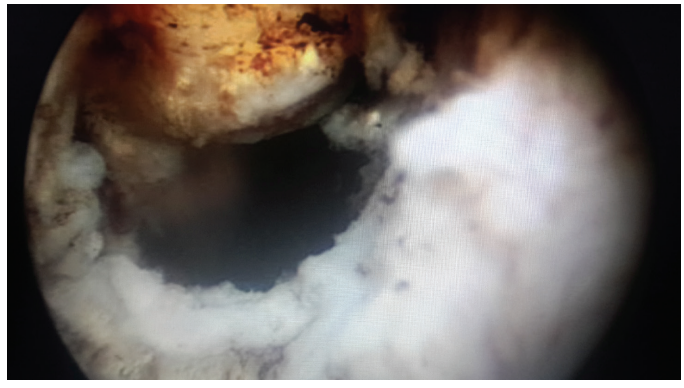

Figure 1. Perforation of the uterus during hysteroscopic myomectomy

A week after the hysteroscopy, the pelvic examination was normal. Four months after the procedure (April 2015), the patient was readmitted to our clinic with delayed menstruation and spontaneous pregnancy was detected (last menstrual period March 12th, 2015). The patient experienced no problems throughout her pregnancy. On December 12th, 2015, a 3124 g 51 cm male infant with the Apgar score 9/10 was delivered by its head in a planned cesarean section. During the exploration, a rupture involving all layers was observed in the area of the previous rupture of the hysteroscopic myomectomy (Figure 2). It was repaired using a double-layer closure with 1.0 absorbable sutures (Figure 3).

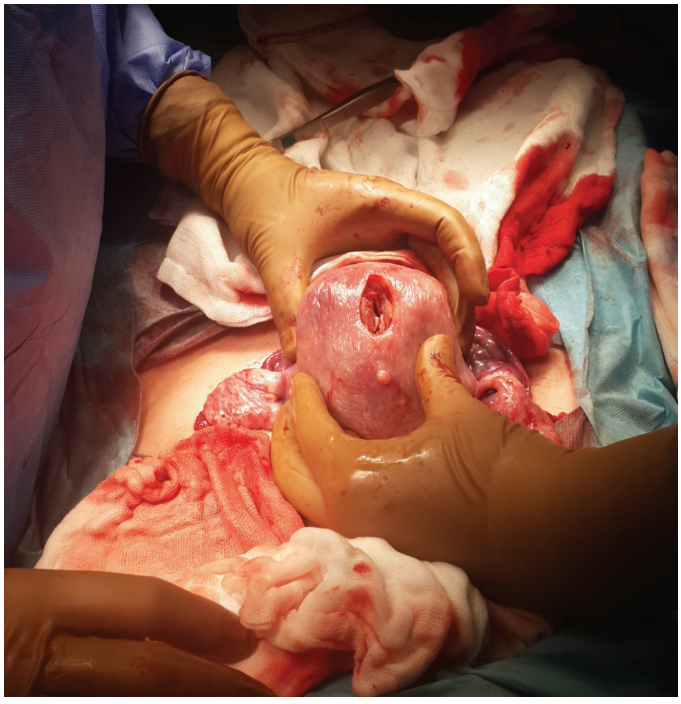

Figure 2. Full thickness, unhealed or scar dehiscence of previous perforation area noticed during elective cesarean section after the baby is delivered

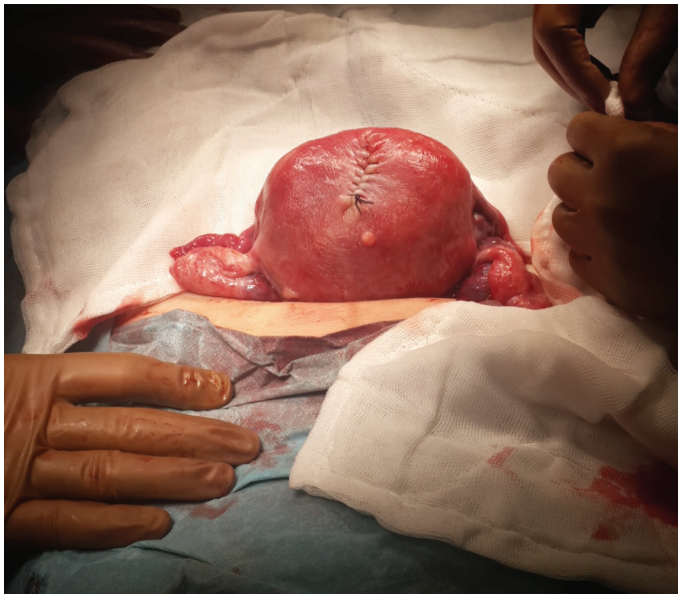

Figure 3. Uterine repair of the defect with two layer sutures

Discussion

Hysteroscopy is a procedure that is commonly used in daily practice for diagnosis and a variety of operations. It is considered as a safe procedure with a low incidence of adverse effects. The rate of complications was reported as 0.28% among 13.600 operations in the Netherlands by Jansen et al.[1] and by Aydeniz et al.[5] as 0.22% among 21.676 hysteroscopies in Germany. Operative hysteroscopy is associated with perioperative and late complications[2].

Uterine rupture can be observed as a perioperative complication and also late in the course of pregnancy. It is a clinically important situation because it can lead to fetal death and maternal morbidity. Uterine perforation and resection are known causes of uterine rupture. Previously reported cases of uterine rupture subsequent to hysteroscopy occurred between weeks 22 and 41. It is most likely to happen in the last weeks of pregnancy but there are reported cases of uterine rupture in the 22nd[6] and 23rd weeks[7] following hysteroscopic metroplasty and fibroid resection, respectively. All of the patients that we have presented became pregnant in a period of less than a year after hysteroscopy. In case 2, the patient had uterine rupture in the 10th week of her pregnancy. According to our literature search, this is the earliest rupture following hysteroscopic septum resection without a complication. Also, in case 3, the week of rupture was 22. Both are early ruptures compared with similar cases in the literature. The short interval between surgery and conception may have cause the early ruptures because no complications occurred in these cases.

According to Propst et al.[8] ,the risks of complications vary according to the type of hysteroscopy. Myomectomy [odds ratio (OR): 7.4] and septum resection (OR: 4.0) had the highest incidence of complications, and diagnostic hysteroscopy (OR: 0.5) and polypectomy (OR: 0.1) had the lowest risks. In case 1, 2, and 3, hysteroscopic septum resection was performed, and in case 4 the hysteroscopic procedure was myomectomy. Both operations are considered as high-risk for complications. Therefore, the type of the hysteroscopy may be a predictive factor. In case 4, the patient underwent myomectomy. During hysteroscopy, a small-sized (7-8 mm) rupture was observed, which was different from the other cases. The patient became pregnant approximately 4 months after surgery, which is a short interval even compared with the patient who had the earliest week of uterine rupture (Case 2). Although she had a previous small rupture, she had no problems during her pregnancy and the rupture was incidentally detected during exploration at cesarean section. This case leads us to two different questions: Did the rupture heal during the four-month-interval and dehiscence occur when the uterus was enlarged because of pregnancy? The second question asks whether it is possible

to become pregnant despite a non-repaired rupture and not experience any complications during pregnancy?

In cases 1 and 4, the uterine rupture was detected late, in the 38th week, and in labor, respectively. The patients delivered healthy babies. The interval was approximately nine months in case 1, and four months in case 4. In cases 2 and 3, the interval was 6 months and the patients experienced uterine rupture early in the course, at the 10th and 22nd weeks.

As a conclusion, the course of uterine rupture in pregnancy after hysteroscopic surgery may depend on many variables, such as the type of surgery, presence or absence of complications such as uterine perforation, the interval between hysteroscopy and pregnancy, and the patient's obstetric history. Uterine rupture may present with abdominal pain and other symptoms, and also can remain silent until delivery when it is detected in exploration. It can happen as late as the last week of pregnancy and as early as the 10th week of pregnancy. Physicians should inform their patients about the possible complications of hysteroscopy and warn them about becoming pregnant in a short time period after surgery because it may increase the risk.

Ethics

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Technical Description / Teknik Açıklama

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Fertility preservation in male patients subjected to chemotherapy; innovative approaches for further progress

Kemoterapiye maruz kalan erkek hastalarda üretkenliğin korunması ve yenilikçi yaklaşımlar

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Abstract

About 4% of male patients with cancer are under the age of 35 years. With the current increase in efficacy and safety of therapies, a growing number of young adults can achieve long-term survival. In male patients receiving systemic chemotherapy and or bone marrow transplantation, a permanent loss of fertility is a common adverse effect. The only possibility to preserve the patient's fertility is to spare the gametes or gamete-forming cells from the chemotherapeutic effect. In adults, this can be achieved by the cryopreservation of spermatozoa with the subsequent application of assisted reproductive technology. Sperm cryopreservation is currently performed using slow-rate cryopreservation as a standard method, in which sperm cells are incubated with a cryoprotective medium and slowly subjected to hypothermia in liquid nitrogen (LN) vapor before they are placed in LN. Another technique called vitrification relies on the direct placement of the cells into LN, after being suspended in a vitrification medium. Many studies compared the clinical outcomes of both techniques and revealed equivalent results. This paper sheds light on some innovative approaches for further progress.

Keywords: Fertility preservation, sperm, testicle, cryopreservation

Öz

Erkek kanserli hastaların yaklaşık %4'ü 35 yaşın altındadır. Tedavilerin etkinliği ve güvenirliği arttıkça artan sayıda genç yetişkinlerin hayatta kalım süresi artmaktadır. Sistemik kemoterapi ve/veya kemik iliği transplantasyonu alan erkek hastalarda doğurganlığın kalıcı olarak kaybı yaygın bir yan etkidir. Hastanın doğurganlığını korumanın tek yolu, kemoterapötik etkiden gametleri veya gamet oluşturan hücreleri korumaktır. Yetişkinlerde, bu, daha sonra yardımcı üreme teknolojisinin uygulanması ile sperm hücrelerinin kriyoprezervasyonuyla elde edilebilir. Sperm kriyoprezervasyonu, şu anda sperm hücrelerinin bir kriyoprotektif ortam ile inkübe edildiği ve yavaşça sıvı azota (LN) yerleştirilmeden önce LN buharında hipotermiye maruz bırakıldığı standart bir yöntem olarak yavaş hızlı kriyoprezervasyon kullanılarak gerçekleştirilir. Vitrifikasyon olarak adlandırılan bir diğer teknik ise, vitrifikasyon ortamına asılarak hücrelerin doğrudan LN'ye yerleştirilmesidir. Pek çok çalışma bu iki tekniğin klinik dönütlerini karşılaştırmıştır. Bu yazı, yapılacak çalışmalara inovatif notlar bırakarak ışık tutmaktadır.

Anahtar Kelimeler: Doğurganlığın korunması, sperm, testis, kriyoprezervasyon

Introduction

About 4% of male cancer patients are under the age of 35 years[1]. With the current increase in efficacy and safety of the therapies, a growing number of young adults can achieve long-term survival[2]. In male patients receiving systemic chemotherapy and or bone marrow transplantation, a permanent loss of fertility is a common adverse effect. The only possibility to preserve the patient's fertility is to spare the gametes or gamete-forming cells from the chemotherapeutic effect. In adults, this can be achieved by the cryopreservation of spermatozoa with the subsequent application of assisted reproductive technology[3].

Sperm cryopreservation is currently performed using slow-rate cryopreservation as a standard method, in which sperm cells are incubated with a cryoprotective medium and slowly subjected to hypothermia in liquid nitrogen (LN) vapor before they are placed in LN. Another technique called vitrification relies on the direct placement of the cells into LN, after being suspended in a vitrification medium. Many studies compared the clinical outcomes of both techniques and revealed equivalent results[3,4].

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Innovative approaches for further progress

1) The role of molecular biology in the sperm cryopreservation

Although most studies that evaluated the cryopreservation of sperm relied on clinical outcomes and the application of cellular biology techniques, current advances in research have revealed much about the molecular biology of the sperm and its role in sperm physiology and fertile ability. Key proteins that can directly affect sperm physiologic parameters have been identified[5].

Prohibitin (PHB) is a 30-kilodalton (kDa) protein that consists of two highly homologous subunits, PHB1 and PHB2, which assemble into a ring-like structure in the mitochondrial inner membrane. The absence of PHB in somatic cells was found to be associated with mitochondrial membrane depolarization and increased generation of reactive oxygen species (ROS). Significant positive correlations were found among PHB expression, mitochondrial membrane potential, and sperm motility in normozoospermia, asthenozoospermia, and oligoasthenozoospermia samples[6]. Together, these observations suggest that PHB expression can be an indicator of sperm quality, and that PHB is important for sperm motility and sperm mitochondrial function.

The focal adhesion kinase protein family appears to have a direct role in protein tyrosine phosphorylation of spermatozoa, which may occur via two pathways, the canonical protein-kinase A pathway and a calcium-stimulated pathway. This protein tyrosine phosphorylation activity is a very important step in the sperm capacitation process, which is required to render the sperm competent to fertilize an oocyte[7]. Accordingly, further progress in the clinical practice of sperm cryopreservation could be achieved through the application of molecular biology techniques in future studies to determine the cryopreservation technique of choice.

Although short-term clinical and cellular biology studies revealed no significant differences between slow cryopreservation and vitrification, the effect on the sperm proteome might have another potential. The Zilli et al.[8] research group used two-dimensional polyacrylamide gel electrophoresis and matrix-associated laser desorption/ionization time-of-flight mass spectrometry to verify whether the protein expression of sea bass sperm was affected by cryopreservation. They stated that the protein profiles differed between fresh and frozen/thawed spermatozoa, as revealed using visual inspection and image analysis software. The group identified 163 spots in fresh sperm; among them, 13 were significantly decreased and 8 were absent in cryopreserved spermatozoa[8].

In addition, the generation of ROS-associated with cryopreservation could be responsible for mammalian sperm damage and the limited value of stored semen in artificial insemination[9]. Increased ROS generation by itself was found to affect human spermatozoa proteins in terms of expression and degradation[10]. A recent study revealed 27 proteins that differed significantly between control and post-thawing human spermatozoa. These proteins are thought to be involved in various sperm physiologic processes, hence, spermatozoa dysfunction after cryopreservation was suggested to be due to protein degradation and or modification[11]. Furthermore, the actin band in western blotting differs between fresh and post-thawing sperm,[6] which might reflect its affection by the process of freezing and thawing, which ultimately affects the functionality and fertilizing ability of the sperm.

Nevertheless, the cryopreservation of swim-up-prepared human spermatozoa with conventional slow freezing and permeating-cryoprotectants-free vitrification showed different degrees of sperm protein affection (Figure 1).

Although no individual proteins were assessed, the application of such a simple molecular biology technique, sSDS-Page (sodium dodecyl sulfate polyacrylamide gel electrophoresis), was able to show significant differences between fresh and post-thawing spermatozoa, as well as between both cryopreservation techniques, regarding the isolated sperm proteins (Figures 1, 2). Of course, further application of mass spectrometry and

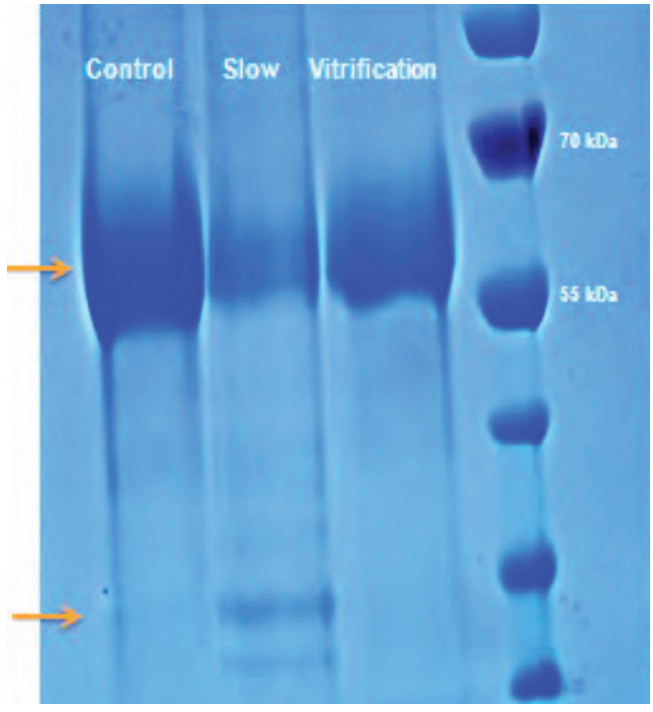

Figure 1. Protein extraction and separation in sodium dodecyl sulfate polyacrylamide gel electrophoresis-page showed different band patterns between control non-frozen, slow, and vitrification post-thawing spermatozoa. The most obvious band in all specimens was a protein band between 55-70 kilodalton. This band is denser in the controls than in vitrification, and denser in vitrification than in slow post-thawing spermatozoa protein extracts. The differences were visible by inspection as well as statistically significant after analysis with image-lab analyzer software ($p<0.05$) (Author's own work)

kDa: Kilodalton

proteomics analysis and or western blotting would provide more precise data about the individual affected proteins and the roles they play in controlling the physiologic parameters and fertilizing ability of sperm. However, such a level of basic and simple investigation was still able to provide reliable evidence that vitrification is superior to conventional slow cryopreservation regarding the degree of affection of sperm proteins, where conventional slow freezing was associated with more significant sperm protein degradation (Figures 1, 2).

Although further progress in this regard is expected soon, sperm cryopreservation can only help post pubertal cancer patients, who are able to provide sperm in one way or another. However, for children exposed to systemic chemotherapy, the cryopreservation of testicular tissue is the only hope for fertility preservation. Established successful cryopreservation of testicular tissue and testicular cell suspensions has been reported with either reimplantation, allowing *in vivo* re-establishment of spermatogenesis, or *in vitro* culture for *ex vivo* spermatogenesis.

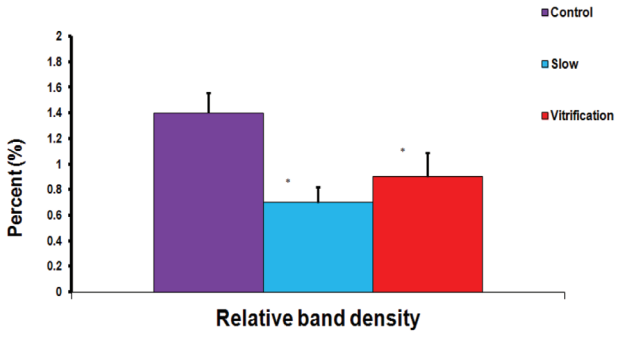

Figure 2. Relative band densities before and after cryopreservation. For the protein band detected in Figure 1, the relative density was decreased from ±1.4 in the controls to ±0.7 in slow freezing (50% reduction, with a significant difference p=0.003), and to ±0.9 in vitrification (36% reduction, with significant difference p=0.009). A significant difference was also found between both cryopreservation techniques (p=0.042). Asterisks indicate significant differences between marked columns with each other as well as with the control (Author's own work)

2) *Ex vivo* testicle perfusion and cryopreservation

Though fertility restoration through testicular tissue cryopreservation has been tested and succeeded in animal models as well as in humans, little is known about the safety aspects and quality of the offspring generations[4]. Here, a new strategy for testicular cryopreservation is described, whose expected advantage over the currently applied techniques of testicular tissue or sperm cryopreservation would be the potential for unrestricted preservation of fertility, dual preservation of fertility and endocrine testicular functions (Table 1),[12] and the preservation of the physiologic route of fertilization, i.e. sexual intercourse, without the application of assisted reproduction technology, which would at least have a significant psychological impact. Moreover, the system allows *ex vivo* testicle perfusion, where high doses of supportive elements and or medications could be supplemented to correct and or improve testicular functions.

The introduced procedure starts with the surgical retrieval of the testicle, where vascular catheterization and immediate perfusion begins. The used perfusates can vary according to the protocol used, for instance, minimal essential medium supplemented with human serum albumen or human tubal fluid medium supplemented with serum substitute supplement (SSS). Further supplementations could be considered according to the protocol used, e.g., ascorbic acid, antioxidants, hormones, growth factors, antibiotics, and heparin.

Immediate and continuous perfusion minimizes the risk of microthrombi formation; however, thrombolytic medications could be supplemented to the perfusates to dissolve any formed microthrombi. At this stage, the testicle has not manifested significant ischemia or oxygen or nutrient deprivation, and the vascular bed is clearly accessible.

Following a short period of *ex vivo* perfusion, the cryopreservation solution (either for slow freezing or for vitrification) can be introduced into the circuit to simultaneously fill the cleaned vascular bed of the testicle and the plastic box around the graft. This ability, together with the presence of temperature adjustors, allow the application of the cryopreservation or vitrification protocol of interest, where at the end, the graft can be stored

Table 1. Hormones produced by the testicles[12]

Hormone	Origin	Regulation	Action
Testosterone	Leydig cells	Gonadotropin-releasing hormone from the hypothalamus causes luteinizing hormone secretion from the pituitary gland, which stimulates the Leydig cells	The control and maintenance of the growth and functions of reproductive organs, libido, and spermatogenesis
Inhibin	Sertoli cells	Gonadotropin-releasing hormone from the hypothalamus causes FSH secretion from the pituitary gland, which stimulates the Sertoli cells	Pituitary feedback inhibition of FSH
Oestradiol	Sertoli cells	Gonadotropin-releasing hormone from the hypothalamus causes FSH secretion from the pituitary gland, which stimulates the Sertoli cells	Produced by testosterone metabolism and may prevent the apoptosis of male germ cells

FSH: Follicle-stimulating hormone

in LN. After the patient's survival, warming protocols could be similarly applied, where the graft can be further perfused till surgical transplantation, minimizing the ischemic reperfusion injury and providing a chance for *ex vivo* testicle reconditioning, using concentrated growth factors and or hormone therapies to omit cryo-injuries before re-implantation (Figure 3).

Materials and methods of author's experimental data

After ethical approval (University of Cologne Nr. 01-106) and patient consents, three semen samples were collected according to the recommendations of the World Health Organization (WHO) from male subjects aged between 25 and 40 years. The samples were collected by masturbation after at least 48 hours of sexual abstinence.

Semen analysis was performed according to the published guidelines of the WHO[13]. Samples were classified according to the following lower reference limits: 15 million spermatozoa/mL, 32% progressive motility, and a minimum of 4% morphologically normal spermatozoa.

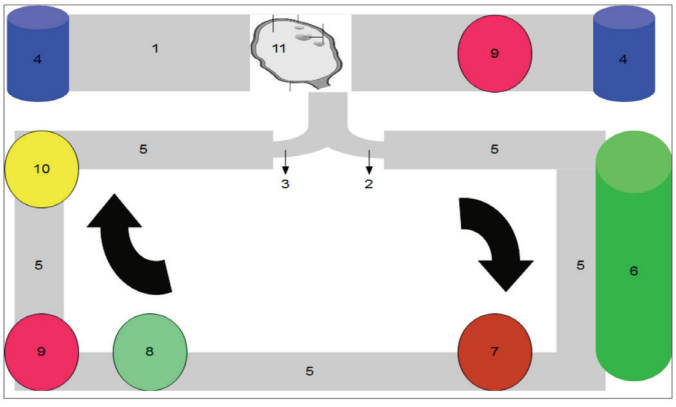

Figure 3. (Author's own innovation)

Diagrammatic representation of the *ex vivo* testicle perfusion system (author's invention):

1. A box to enclose the testicle that works as a sealed cryovial
2. Testicular vein stump connected to the circuit using a special cannula or catheter (perfusion output)
3. Testicular artery stump connected to the circuit using a special cannula or catheter (perfusion input)
4. Reservoirs for filling the testicle-containing box with medium, the cryoprotective, vitrification, and warming solutions during cryopreservation
5. The perfusion circuit
6. Perfusate reservoir
7. Centrifugal pump (pulsatile or continuous flow)
8. Set of leukocytic and cytokines filters
9. Temperature adjustor
10. A gas exchanger to remove carbon dioxide and provide oxygen to maintain these gases in the perfusate at physiologic levels
11. The testicle subjected to perfusion and cryopreservation

Each semen sample was diluted 1:2 with pre-warmed (37 °C) Quinn's Sperm Wash Medium (Sage Media, Trumbull, CT, USA) and transferred into a conical centrifuge tube (Becton Dickinson, NJ, USA) and centrifuged at 300 g for 10 minutes. The supernatant was carefully removed and discarded. The sperm pellet was resuspended in 1 mL of the same medium by gentle pipetting, followed by centrifugation again for 10 minutes at 300 g. After removing and discarding the supernatant, 1 mL of pre-warmed (37 °C) human tubal fluid medium +1% SSS was gently placed over the pellet, without disturbing it, followed by incubation for 60 minutes, at 37 °C in a 6% CO_2 atmosphere, in the oblique position (45 °C). After incubation, the tube was handled gently and returned to the up-right position and the uppermost 500 µL medium, where the highly motile sperms are present, was removed into a sterile Eppendorf tube[13].

Each swim-up preparation was divided into three equal parts; one was the fresh control, one for subsequent conventional slow freezing, and one for subsequent vitrification. Slow freezing and vitrification were performed according to the guidelines[13].

Protein extraction and SDS polyacrylamide gel electrophoresis

Fresh, slow cryopreserved and vitrified spermatozoa of the same sample and concentration were centrifuged at 300 g for 10 minutes. The supernatant parts were removed and discarded, while the cellular pellets were resuspended in 100 µL radioimmunoprecipitation assay lysis buffer supplemented with a 10% animal component-free protease inhibitor cocktail (Sigma, Munich, Germany), with vigorous shaking, vortex and sonication, when needed, to disrupt the pellet.

The protein concentration and protein amount in each sample were determined using the Bradford method. A standard curve was obtained using blank water and serial protein concentrations of 50-1600 µg/mL of bovine serum albumin. After duplicates of serial dilutions of each sample (6 µL) were equilibrated with color reagent (100 µL) (Bio-Rad Laboratories GmbH, München, Germany) for 10 minutes at room temperature, the absorbance measurements were made using a double-beam ultraviolet-visible spectrophotometer. The standard curve was plotted and the protein concentration in each sample was determined relative to the standard curve.

Equal amounts of protein were subjected to SDS-Page together with 10 µL of pre-stained page ruler protein marker (10-170 kDa) (Thermo Fisher Scientific, Bonn, Germany). The separated protein bands were stained in the gel using Coomassie blue (Thermo Fisher Scientific, Bonn, Germany). The stained gels were then scanned and the densities of the bands were determined using image-lab analyzer software (Life Science Research, Bio-Rad, München, Germany).

The relative band densities were calculated by dividing the actual band density (obtained by the image-lab analyzer) by the average of the three bands of each sample (control and post-thawing bands). The results were detectable visually, as well as with statistical calculations. For statistical analysis, an Excel data

sheet (Microsoft Office 2007) for the calculation of mean and standard deviation was used. A comparison between the three treatments was performed using the Prism 6 Demo program for the determination of significant differences using the non-paired t-test, where p values less than 0.05 were considered significant.

Ethics

Ethics Committee Approval: The study was approved by University of Cologne Ethical Committee (approval number: 01-106).

Informed Consent: Consent was taken by the patients.

Peer-review: External and internal peer-reviewed.

Conflicts of interest: The intellectual properties and the system included in this manuscript belong solely to the author. All rights are preserved solely for the author. Reproduction or use of any of the included intellectual properties requires the written permission of the author.

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The significance of reverse flow in ductus venosus between sixteen and twenty weeks' gestation

On altıncı ve yirminci gebelik haftaları arasında değerlendirilen duktus venozus ters akımının önemi

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