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Clinical Investigations

TURKISH SOCIETY OF OBSTETRICS AND GYNECOLOGY

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Turkish Journal of Obstetrics and Gynecology (former 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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Cover letter to the editors addressing the following points:

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· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

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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/),

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

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

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

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.

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

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Precis

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

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

Abstract

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

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

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

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

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

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

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

Keywords

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

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

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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 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.

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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.

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Examples

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Book; Arici A, Seli E. Non-invasive Management of Gynecologic Disorders. In: Arici A, Seli E (eds). London: Informa Healthcare; 2008.

Tables and Figures

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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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Colleagues,

Following the 25th European Congress of Obstetrics and Gynaecology in conjunction with 15th Congress of Turkish Society of Obstetrics and Gynecology, that was held last week in Antalya, we would like to take this opportunity to thank you very much once again for your participation and your invaluable contribution to the congress.

This year's congress was a great accomplishment, with an attendance of more than 2,064 colleagues from 36 different countries. We diversified the scientific programme with 5 keynote lectures, 68 plenary sessions, 7 international sessions, 12 master classes, 7 hot courses, 13 oral presentation sessions and 7 industry sponsored symposia on different topics with interactive discussions and hands-on applications. The scientific sessions were great platforms for effective discussions.

The positive feedback we have received from hundreds of participants has made us very glad and proud. This congress would have not been so successful without your precious support. Thank you again for all your time, commitment and effort.

Another gladsome news has arrived that our journal, the "Turkish Journal of Obstetrics and Gynecology" has been accepted to PubMed Central. I would like to thank Eray Çalışkan and all the members of Editorial Board of the journal for their extraordinary effort and precious contributions. Our journal will be much stronger with the vision of becoming a worldwide renowned journal, with your supports and contributions.

With my kind regards,

Sincerely
Ateş Karateke, Prof. MD
President of TSOG

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

I am glad to announce that our journal "Turkish Journal of Obstetrics and Gynecology" is now accepted to be indexed in "PubMed Central". PubMed is accessed for free and have been shown to be the optimum research tool for biomedical journals[1]. Being indexed in PubMed increases the visibility of the manuscripts and the probability of being read/cited by researchers in the field. Also, studies showed that publishing the manuscript in an open access journal increases its probability of its citation. Open access articles compared to non-open access articles remained twice as likely to be cited [odds ratio: 2.1 (1.5-2.9)] in the first 4-10 months after publication, with the odds ratio increasing to 2.9 (1.5-5.5) 10-16 months after publication[2].

Turkish Journal of Obstetrics and Gynecology has now bared both the advantage of an open journal and visibility in PubMed Central, besides its vigorous editorial work and double blind reviewer system. From now on we will focus on increasing our impact factor through publishing high quality research conducted in an ethical set-up.

We now call for well conducted high quality research. Submit your articles for fast, fair, scientific and ethical review system.

Best wishes

Eray Çalışkan, Editor

References

1. Falagas ME, Pitsouni EI, Malietzis GA, Pappas G. Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. FASEB J 2008;22:338-42.
2. Eysenbach G. Citation advantage of open access articles. PLoS Biol 2006;4:e157.

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2017;14:76-81

Outcome of intracytoplasmic sperm injection after preinstillation of a gonadotropin releasing hormone agonist in the uterine cavity just before embryo transfer

Embriyo transferi öncesi uterin kaviteye gonadotropin serbestleştirici hormon agonisti enjeksiyonunun intrasitoplazmik sperm enjeksiyonu sonuçları

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Abstract

Objective: To evaluate the effects of a gonadotropin releasing hormone agonist (GnRHa) injection prior to embryo transfer on implantation and pregnancy rate.

Materials and Methods: We performed a retrospective analysis of patients undergoing *in vitro* fertilization (IVF) therapy with and without GnRHa preinstallation into the uterine cavity just before embryo transfer between January 2012 and March 2013 in a single IVF center of a university hospital. Patients were evaluated based upon implantation, pregnancy, live birth, and miscarriage rates.

Results: GnRHa was injected into the uterine cavity of 108 patients prior to embryo transfer which were regarded as study group. One thousand forty-seven patients who were not injected GnRHa were regarded as the control group. Pregnancy rates were 44.4% and 41.7% in the GnRHa and control groups, respectively. Live birth rates were 27.8% and 26.1%, miscarriage rates were 15.7% and 15.7%, and implantation rates were 31% and 30%, respectively and there were no difference between groups statistically ($p>0.05$).

Conclusion: No statistically significant differences in implantation, pregnancy, live birth, or miscarriage rates were observed in patients treated with GnRHa prior to embryo transfer, relative to the controls. Therefore, GnRHa injection into the uterine cavity prior to embryo transfer is not recommended as a means of increasing implantation or pregnancy rates in IVF. However, prospective randomized controlled studies are needed to clarify the effect of GnRHa instillation in the uterine cavity for embryo implantation in IVF.

Keywords: Gonadotropin releasing hormone agonist, embryo transfer, uterine cavity, *in vitro* fertilization

Öz

Amaç: Embriyo transferi öncesi uterin kaviteye gonadotropin serbestleştirici hormon agonisti (GnRHa) enjeksiyonunun implantasyon ve gebelik oranlarına etkisini değerlendirmektir.

Gereç ve Yöntemler: Ocak 2012-Mart 2013 tarihleri arasında üniversite hastanemizin *in vitro* fertilizasyon (IVF) kliniğinde IVF tedavilerinden sonra ve embriyo transferi öncesinde uterin kaviteye GnRHa enjeksiyonu uygulanan ve uygulanmayan hasta grupları retrospektif olarak tarandı. Her iki grubun implantasyon, gebelik, canlı doğum ve düşük oranları karşılaştırıldı.

Bulgular: Bu tarihler arasında uterin kaviteye embriyo transferi öncesi GnRHa enjekte edilen 108 hasta çalışma grubumuz olarak belirlenirken, bu süreçte GnRHa enjekte edilmemiş 1047 hasta kontrol grubu olarak kabul edildi. İmplantasyon oranlarına baktığımızda sırasıyla GnRHa ve kontrol grubunda %31 ve %30, gebelik oranları %44,4 ve %41,7, canlı doğum oranları %27,8 ve %26,1 ve düşük oranları %15,7 ve %15,7 olarak saptanmış ve gruplar arasında fark görülmemiştir.

Sonuç: Uterin kaviteye embriyo transferi öncesi GnRHa enjeksiyonunun istatistiksel olarak, implantasyon, gebelik, canlı doğum ve düşük oranlarına bir etkisi bulunmamıştır. Bu nedenle, tüp bebek uygulamalarından sonra embriyo transferi öncesi uterin kaviteye GnRHa enjeksiyonu önerilmemektedir. Fakat bu konuda prospektif randomize çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Gonadotropin serbestleştirici hormon agonisti, embriyo transferi, uterin kavite, *in vitro* fertilizasyon

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PRECIS: Gonadotropin releasing hormone agonist instillation into the uterine cavity before embryo transfer is not recommended to increase implantation and pregnancy rate in ***in vitro*** fertilization.

Introduction

The success of *in vitro* fertilization (IVF) as a means of achieving clinical pregnancy is primarily dependent on the presence of high quality embryos and a suitable endometrium for implantation. Although the definition of high quality embryos is well established based upon morphologic criteria of the cleavage embryo or blastocyst, there remains no widely accepted morphologic or structural criterion that is used to assess the suitability of the endometrium. Therefore, much of the research in IVF has been focused on improving implantation rates through processes such as luteal phase support, which is now strongly recommended in IVF[1].

Progesterone, estradiol, human chorionic gonadotropin (hCG), or gonadotropin releasing hormone agonists (GnRHa) have been evaluated in a variety of clinical studies as a means of luteal phase support to increase implantation and pregnancy rates in IVF[2-5]. More recent studies have suggested that intrauterine injections of hCG just before embryo transfer (ET) can increase pregnancy rates[2,6,7]. In cases of thin endometrium and recurrent pregnancy loss, injection of filgrastim (granulocyte colony-stimulating factor) into the uterine cavity is suggested as a means of increasing implantation and pregnancy rates[8,9].

It has been hypothesized that the local expression of GnRH and its receptor may play a role in endometrial conditioning during implantation because GnRH receptors are present in the follicles, embryo, placenta, tubal epithelium, and endometrium[10-14]. Similarly, Casan et al.[12] hypothesized that GnRH receptors in the tubal epithelium may play a role in fertilization, early embryonic development, and implantation during the early luteal phase via a combination of paracrine and autocrine methods. Here, we compared the effects of GnRHa injected directly into the uterine cavity prior to ET on implantation rate, pregnancy, and live birth rates. This is the first study in the literature of GnRHa pre-installation before ET to investigate improvements in implantation and pregnancy rates in IVF.

Materials and Methods

In our study, we evaluated the effects of GnRHa injection prior to ET on implantation and pregnancy rates in our IVF programme executed between January 2012 and March 2013. Outcomes of IVF cycles during that period were collected retrospectively. Injection of GnRHa into the uterus before ET was a standard treatment protocol used by a single physician (BH) in our clinic during that period. Outcomes of IVF cycles treated with GnRHa were compared with those that received IVF treatment without GnRHa. Informed consent was obtained from all patients prior to the initiation of any procedure. This study was approved by the Institutional Review Board of Başkent University, Ankara, Turkey (approval number: KA 15/302).

Only fresh cycles in which ET was performed were included. In addition, only the cycles of patients who were aged ≤40 years at the time of treatment, whose embryos were grade 1 or 2, and whose endometrial thickness was ≥7 mm on the day of hCG were included. The exclusion criteria included cycles comprising difficult ET, cancellations for other reasons, and patients who had 3 or more previous unsuccessful IVF cycles. A total of 1965 consecutive IVF cycles were performed in our clinic between January 2012 and March 2013, among which 142 cycles received GnRHa injection before ET. From this cohort of IVF cycles, 1155 met the necessary inclusion criteria, including 108 who received GnRHa injection before ET and 1047 that did not. Primary outcomes in the two groups were implantation and live birth rates, and secondary outcomes were pregnancy and miscarriage rates.

Ovarian hyperstimulation and oocyte pick up

Ovarian hyperstimulation was performed according to the long protocol, antagonist protocol, or clomiphene citrate-follicle stimulating hormone (CC-FSH) protocol. In the long protocol, downregulation was achieved using GnRH analogues initiated at the start of the proceeding luteal phase, with dosing halved beginning on the day of hyperstimulation and continued until the day of hCG treatment. The patients were then treated with recombinant FSH in the early follicular phase. In the antagonist protocol, FSH analogues were started at the beginning of the follicular phase of the cycle, and GnRH antagonists were added when the leading follicles reached 12-13 mm in diameter. In the CC-FSH protocol, patients were treated with 50 mg clomiphene citrate twice a day beginning on the third day of the cycle, and augmented with FSH on the third day of CC treatment. In all protocols, ovulation was induced using 250 µg recombinant hCG that was administered after the leading follicle reached a minimum of 17 mm in diameter.

Oocyte retrieval was performed at 32-34 h after hCG administration. Oocytes were collected transvaginally. Metaphase II (MII) oocytes were prepared for the intracytoplasmic sperm injection (ICSI) procedure, which was performed after 2-2.5 h incubation. Embryos were transferred at the cleavage stage (3 days after ICSI).

Embryo grading

Embryos were graded according to the number and equivalence of cells and the percentage of vacuoles formed around them, the presence of multinucleation, and the status of the zona pellucida on the second and third days. According to the morphologic criteria above, embryos were graded as 1-4 on the cleavage stage embryo. According to Turkish legal regulations, patients aged younger than 35 years can only receive one embryo, and two embryos can be transferred in patients aged 35 years or older, as well as in patients who have already undergone two unsuccessful ICSI cycles. Thus, one or two embryos were transferred on the third day after fertilization.

Gonadotropin releasing hormone agonist injection and embryo transfer

For patients receiving GnRHa treatment, the vaginal cavity was washed with warm saline and embryo medium, followed by treatment with 0.5 mL GnRHa (including 0.1 mg/mL triptoreline acetate, Ferring, Greece), which was delivered via an embryo catheter under abdominal ultrasonography guidance. GnRHa was injected into the uterine cavity when the tip of the catheter reached 1 cm below the inner cavity of uterine fundus. Pre-selected embryos were then transferred into the uterine cavity after 10 min.

Luteal support

All patients received luteal support, including 90 mg/day progesterone administered intra-vaginally starting on the day of ET and continued for 12 days.

Serum hCG levels were analyzed on day 12, with levels >10 mIU/mL suggestive of pregnancy. Clinical pregnancy was accepted as positive if the fetal gestational sac was apparent and a fetal heartbeat was regularly detected. The implantation rate was calculated as the number of gestational sacs seen on transvaginal ultrasound per number of transferred embryos. Miscarriage rates were calculated as the number of miscarriages before 12 weeks of gestation per cycles of ET. The live birth rate was calculated as the number of live births per IVF cycles with ET.

Statistical Analysis

Demographic data for each group are presented as the mean ± standard deviations, or the median, with minimum and maximum or percentage values. The chi-square test was used to compare categorical data, and the Mann-Whitney U test was used to compare continuous variables that were non-normally distributed. Student's t-test was used to compare continuous data with normal distribution. P values <0.05 were considered statistically significant. Data were analyzed using SPSS version 18.0 for Windows (SPSS, Inc., USA).

Results

After the exclusion of 34 cycles from the study and 474 cycles from the control groups, 108 IVF cycles formed the study and 1047 IVF cycles formed the control groups. Thirty-four cycles in the GnRHa group were excluded from the study.

Patient characteristics including age, duration of infertility, bilateral ovarian antral follicle counts on the day of early

Table 1. Characteristics of study groups

	GnRHa (+) n=108	Control group n=1047	p
Female age (y)	30.4±4	31.2±4	0.10
Infertility duration (y)	5.7±4	5.8±4	0.78
Antral follicle (n)	5.6±2	5.1±2	0.06
Estradiol level on hCG day (pg/mL)	1631±947	1561±1068	0.51
Progesterone level on hCG day (ng/mL)*	0.45 (0.1-1.9)	0.40 (0.0-2.2)	0.21
Endometrial thickness on hCG day (mm)	11.1±2.1	10.8±2.0	0.07
Ovarian hyperstimulation duration (d)	9.0±1	9.0±2	0.90
Total gonadotropin doses (IU)	1987±695	2009±760	0.79
Total oocytes after OPU (n)	12.2±5.6	10.9±6.2	0.12
MII oocyte (n)	9.9±5	8.4±5	0.09
Transferred embryo (n)	1.2±0.4	1.2±0.4	0.86
Grade 1 embryo (n)*	0.3 (0-2)	0.3 (0-2)	0.21
Grade 2 embryo (n)	0.8±0.6	0.9±0.6	0.17
IVF reasons (%)			0.87
Male factor	34.5	35.1	
Unexplained	22.1	25.6	
Anovulation	26.3	24.3	
Poor ovarian reserve	8.3	9.8	
Endometriosis	5.2	5.3	
Tubal factor	2.8	3.0	

p-values according to the $\chi 2$ test for categorical data

*Student's t-test for normally distributed data or the Mann-Whitney U test for non-normally distributed quantitative data

Values were means ± standard deviation and medians for non-normally distributed quantitative data

GnRHa: Gonadotropin releasing hormone agonist, n: Number, y: Year, d: Day, IU: International units, hCG: Human chorionic gonadotropin, OPU: Oocyte pick-up, MII: Metaphase II, IVF: ***In vitro*** fertilization

follicular phase, estradiol and progesterone levels, endometrial thickness on the day of hCG injection, duration of ovarian hyperstimulation, total dose of gonadotropins used for ovarian stimulation, number of total oocytes and MII oocytes obtained on the oocyte pick-up day, grade of the embryos, number of transferred embryos, and the reasons of IVF cycles were similar in both groups (Table 1).

Ovarian hyperstimulation was achieved using the long, agonist, or CC-FSH protocols. The long protocol was used in 23.1% and 25% of cycles for the GnRHa and controls groups, respectively. The agonist protocol was used for 74.1% and 70.6% of cycles, and the CC-FSH protocol was used in 4.4% and 2.8% of cycles in the GnRHa and control groups, respectively (p=0.63).

Pregnancy, live birth, miscarriage, and implantation rates were 44.4%, 27.8%, 15.7%, and 31%, respectively, in the GnRHa group compared with 41.7%, 26.1%, 15.7%, and 30%, respectively, in the control group. No statistically significant differences were observed between the two groups in terms of IVF results (Table 2). Similarly, the transfer of one or two embryos did not affect pregnancy, live birth, or implantation rates in the GnRHa group relative to the controls (p>0.05) (Table 3).

Discussion

In this study, we compared pregnancy-related IVF outcomes in patients treated with GnRHa immediately before ET with those receiving similar treatment without GnRHa stimulation. We observed no statistically significant differences in terms of implantation, pregnancy, live birth, or miscarriage rates between the groups.

During IVF treatment, spontaneous pregnancy is encountered in 1% of cycles using the long protocol when GnRHa is started during the previous luteal phase, consistent with an observation that inadvertent GnRHa injection might improve pregnancy rates[15]. Luteal phase injection of GnRHa has been suggested as a method to increase pregnancy and live birth rates by enhancing luteinizing hormone secretion and supporting maintenance of the corpus luteum[5,16,17]. Alternatively, GnRHa is also thought to affect GnRH receptors in the endometrium and embryo, thereby increasing implantation and pregnancy rates in donor cycles[5]. Based upon these observations, GnRHa injection directly into the uterine cavity has been suggested as a possible method for enhancing implantation rate, although no direct analyses have been performed.

Previous studies have offered strong support for the use of intrauterine therapy as a means of improving IVF outcomes. In a series of prospective, randomized controlled studies, hCG injection into the uterine cavity before ET increased pregnancy and live birth rates[2,6,7]. Under normal conditions, trophoblastic GnRH has been implicated as one of the primary regulators of the synthesis and secretion of hCG in peri-implantation embryos in murine models. Similarly, human placental GnRH plays an important role in the synthesis and secretion of hCG[11,18].

Table 2. *In vitro* fertilization outcomes of the two groups

	GnRHa (+) n=108	Control group (-) n=1047	p
Pregnancy rate/cycle n (%)	48/108 (44.4)	437/1047 (41.7)	0.32
Live birth rate/cycle n (%)	30/108 (27.8)	273/1047 (26.1)	0.38
Miscarriage rate/cycle n (%)	17/108 (15.7)	164/1047 (15.7)	0.36
Implantation rate/cycle (%)	31.2±44	30.7±43	0.86

p-values according to the χ^2 test for categorical data
Student's t-test for normally distributed data
GnRHa: Gonadotropin releasing hormone agonist, n: Number

Table 3. Outcomes of *in vitro* fertilization according to the number of transferred embryos

	GnRHa (+)	Control group	p
One embryo transfer			
Pregnancy rate n (%)	34/79 (43.0)	304/750 (40.5)	0.37
Live birth rate n (%)	20/79 (25.3)	191/750 (25.5)	0.55
Miscarriage rate n (%)	14/79 (17.8)	113/750 (15.2)	0.13
Implantation rate (%)	32±4.7	34±4.7	0.83
Two embryo transfer			
Pregnancy rate n (%)	14/29 (48.3)	133/293 (45.4)	0.45
Live birth rate n (%)	10/29 (34.5)	82/293 (28.0)	0.29
Miscarriage rate n (%)	4/29 (13.8)	51/293 (17.4)	0.83
Implantation rate (%)	27±34	22±31	0.38

p-values according to the χ^2 test for categorical data
Student's t-test for normally distributed data
GnRHa: Gonadotropin releasing hormone agonist, n: Number

Alternatively, GnRHa may also increase pregnancy rates by increasing the synthesis of hCG in the endometrium. However, despite these observations, the precise mechanism by which GnRHa improves pregnancy rates has remained unknown. Finally, *in vitro* studies investigating the effects of GnRHa during cleavage and implantation will be necessary to fully understand the mechanisms on GnRHa activity.

Study Limitations

The most important limitation of our study is that only a single physician used GnRHa injections, so we cannot rule out the selection or performance bias in the follow-up of IVF treatment. The retrospective nature of this analysis represents the second significant limitation of this work; however, we tried to minimize both limitations by strictly controlling the inclusion and exclusion criteria. Patients with thin endometriums were excluded from the study; although there are studies suggesting the opposite[19], most authors emphasize that pregnancy rates were lower in patients with thin endometriums[20-22]. Similarly, the exclusion of patients aged >40 years was preferred to limit the effects of embryo quality on implantation. Only first or second IVF cycles of the same patient were included in this analysis to minimize the effects of recurrent implantation failure and to exclude the recurrent number of IVF cycles of the same patient. Finally, only cycles in which grade 1 or 2 embryos were transferred were included as a means of controlling for the effects of embryo quality.

Conclusion

No statistically significant differences in terms of implantation, pregnancy, live birth, and miscarriage rates were observed in patients treated with GnRHa prior to ET relative to the controls. According to this study, GnRHa injection into the uterine cavity prior to ET is not recommended as a means of increasing implantation or pregnancy rates in IVF. However, in order to be able to see the net effect of GnRHa injection into the uterine cavity before ET in IVF, further prospective randomized controlled studies are needed.

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Ethics

Ethics Committee Approval: The study was approved by Başkent University Ethical Committee (approval number: KA 15/302).

Informed Consent: Because of restrospective nature of the study, informed consent was not needed to be taken fron the patients.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Day 3 embryo transfer versus day 5 blastocyst transfers: A prospective randomized controlled trial

Üçüncü gün embriyo transferlerinin 5. gün blastokist transferleriyle karşılaştırılması: Prospektif, randomize, kontrollü bir çalışma

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Abstract

Objective: This study aimed to show whether transferring day 5 embryos resulted in higher implantation and pregnancy rates than transferring day 3 embryos in Turkish women undergoing an intracytoplasmic sperm injection (ICSI) cycle.

Materials and Methods: A total of 190 women who had ICSI after retrieval of more than four oocytes on the day of fertilization check were randomly assigned to undergo embryo transfer either on day 3 or day 5.

Results: Day 3 and day 5 transfers were statistically similar with respect to the age of woman (p=0.107), duration of infertility (p=0.528), cause of infertility (p=0.850), number of collected oocytes (p=0.119), number of metaphase II oocytes (p=0.178), number of fertilized oocytes (p=0.092), and number of transferred embryos (p=0.556). The number of grade 1 embryos was significantly higher in day 5 transfers than in day 3 transfers (p=0.001). Day 3 and day 5 embryo transfers had statistically similar implantation, clinical pregnancy, twinning, and live birth rates (p=0.779, p=0.771, p=0.183, and p=0.649, respectively). The live birth rates in singleton pregnancies conceived after day 3 and day 5 embryo transfers were statistically similar (p=0.594).

Conclusion: The efficacy of blastocyst transfer is not inferior to that of embryo transfer on cleavage stage. Performing blastocyst transfer may have benefits because it is associated with acceptable pregnancy rates and morphologic assessment on day 3 has limited predictive value for subsequent embryonic development.

Keywords: Blastocyst, embryo transfer, *in vitro* fertilization, intracytoplasmic sperm injection

Öz

Amaç: Bu çalışma, intrasitoplazmik sperm enjeksiyonu (ICSI) uygulanması planlanan Türk kadınlarında 5. gün blastokist transferinin 3. gün embriyo transferine göre daha yüksek implantasyon ve gebelik oranlarıyla sonuçlanıp sonuçlanmadığını belirlemeyi amaçlamaktadır.

Gereç ve Yöntemler: Fertilizasyon kontrol gününde en az dört fertilize oosit toplanan ve ICSI yapılması planlanan 190 kadın, randomize olarak iki gruba ayrılmış; bir gruba 3. gün embriyo transferi yapılırken diğer gruba 5. gün blastokist transferi uygulanmıştır.

Bulgular: Embriyo ve blastokist transferi yapılan kadınlar; yaş (p=0,107), infertilite süresi (p=0,528), infertilite nedeni (p=0,850), toplanan oosit sayısı (p=0,119), metafaz II oosit sayısı (p=0,178), fertilize oosit sayısı (p=0,092) ve transfer edilen oosit sayısı (p=0,556) bakımından benzerdi. Embriyo transferlerine göre blastokist transferlerinde sınıf 1 embriyo sayısı anlamlı olarak yüksekti (p=0,001). Embriyo ve blastokist transferi yapılan kadınlar, benzer implantasyon, klinik gebelik, ikiz gebelik ve canlı doğum oranlarına sahipti (sırasıyla p=0,779, p=0,771, p=0,183 ve p=0,649). Embriyo ve blastokist transferi sonucu elde edilen tekiz gebeliklerde de canlı doğum oranları benzerdi (p=0,594).

Sonuç: Blastokist transferinin embriyo transferine göre daha az etkin olmadığı görülmüştür. Tatmin edici gebelik oranlarıyla sonuçlandığı için ve 3. günde gerçekleştirilen morfolojik embriyo değerlendirmesinin ileriye dönük öngörü gücü kısıtlı olduğundan, blastokist transferi yapmak daha yararlı olabilir.

Anahtar Kelimeler: Blastokist, embriyo transferi, *in vitro* fertilizasyon, intrasitoplazmik sperm enjeksiyonu

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PRECIS: Blastocyst transfer is an efficient embryo transfer method associated with satisfactory pregnancy outcomes, and performing blastocyst transfer may have benefits because morphologic assessment on day 3 has limited predictive value for subsequent embryonic development.

Introduction

Intracytoplasmic sperm injection (ICSI) traditionally refers to the process of fertilization by combining an egg and sperm in the laboratory and then transferring the obtained embryos to the uterus. Embryo transfer (ET) performed on the second or third day of fertilization when the embryos are at the 4-8 cell stage has an implantation rate lower than 20%[1-3].

In Turkey, ET is performed according to the regulations of the Ministry of Health. Single ET (SET) is recommended in women aged younger than 35 years who are having their first or second ET, whereas a maximum of two embryos can be transferred to women older than 35 years who have had 2 previous unsuccessful embryo transfers[4]. This restriction on ET reveals the need to increase the efficiency of *in vitro* fertilization (IVF) cycles. It has been hypothesized that blastocyst stage ET would permit the identification of embryos that could maintain their developmental process and, thus, allow the recruitment of good quality embryos with enhanced developmental ability. Therefore, it has been suggested that the blastocyst transfer at day 5 would succeed the highest pregnancy rates with the least number of embryos[5-7]. It is well known that growth and blastocysts at the 8-cell stage need a more complex environment, but the recent formulation of highly specialized media has permitted the preservation of blastocysts *in vitro*[8-10].

The present study was arranged to determine whether transferring blastocyst-stage embryos would result in higher implantation and pregnancy rates than transferring cleavage-stage embryos in Turkish women undergoing ICSI cycles within the context of legal restrictions on the number of embryos being transferred. The findings of this study would correspond to the necessity of data regarding the implementation of blastocyst transfer in elective SET or double ET.

Materials and Methods

This prospective randomized study was conducted in accordance with the ethics principles outlined in the Declaration of Helsinki and approved by the local ethics committee (approval number: 149.01.2016).

Patient selection and randomization

A total of 218 patients from whom at least four fertilized oocytes were retrieved on day 1 of fertilization (pronuclear scoring) during an ICSI cycle were eligible. The retrieval of at least four fertilized oocytes was set up as a criterion for eligibility to keep the risk for treatment discontinuation and cycle cancellation at a minimum. Women who were eligible were informed about the study. After the exclusion of 17 patients who refused to participate in the study, the remaining 201 patients gave written informed consent and were then randomized into either the day 3 embryo or day 5 transfer groups (n=100, n=101, respectively) using a computer-generated random number list. Once a patient was randomized, she remained in the same group throughout the study. Seven patients who discontinued their ICSI cycle due to developmental arrest at blastocyst stage and four patients who were thought high-risk for ovarian hyperstimulation syndrome were excluded from the study. Therefore, 95 patients in the day 3 ET group and 95 patients in the day 5 ET group were included in the final analysis (Figure 1).

Controlled ovarian hyperstimulation and oocyte retrieval

The main stimulation protocol used was the long protocol. All patients receive leuprolide acetate (Lucrin®, Abbott Pharmaceuticals, IL, USA) on day 21 of the menstrual cycle. When menstrual bleeding began, transvaginal ultrasonography was performed and serum estradiol concentration was measured. As soon as ovarian quiescence was provided with the absence of ovarian cysts, and estradiol levels were below 50 pg/mL, the administration of gonadotropins was initiated with recombinant follicle-stimulating hormone at a daily dose of 150 IU (Gonal F®, Serono Laboratories, Randolph, MA, USA). The daily dose was adjusted by individual response. When at least three follicles reached a mean diameter of 17 mm, human chorionic gonadotropin [(HCG), Ovitrelle®, Merck-Serono, Aubonne, Switzerland] was administered. About 35-36 hours later, oocytes were retrieved using a double-lumen aspiration needle (Swemed Laboratories, Billdal, Sweden) under transvaginal ultrasonography guidance.

The follicular aspirate was poured into 60 mm Falcon dishes (Beckton Dickinson Labware, Franklin Lakes, NJ, USA) and cumulus-oocyte complexes were transferred into another dish with standard IVF medium (MediCult, Jillinge, Denmark). After the evaluation of each cumulus-oocyte complex for cumulus-corona cell morphology, the complexes were incubated in standard incubators until the time of removal of cumulus oophorus and corona radiata cells for ICSI.

Sperm preparation

All semen samples liquefy for 15 to 30 minutes in an incubator. Sperm was prepared using a Percoll gradient (95 an 47.5%; Sigma, St Louis, MO, USA). One-half to 2 mL of raw semen was layered over the Percoll and the preparations were centrifuged at 300 g for 20 minutes. After centrifugation, the pellets from each tube were collected into 5 mL of culture medium and centrifuged at 1800 g for ICSI for 10 minutes. Fertilization was confirmed by the presence of two pronuclei and two polar bodies on day 1.

Embryo grading

Embryos for day 3 transfer were cultured in the standard culture medium, whereas blastocysts for day 5 transfer were moved into

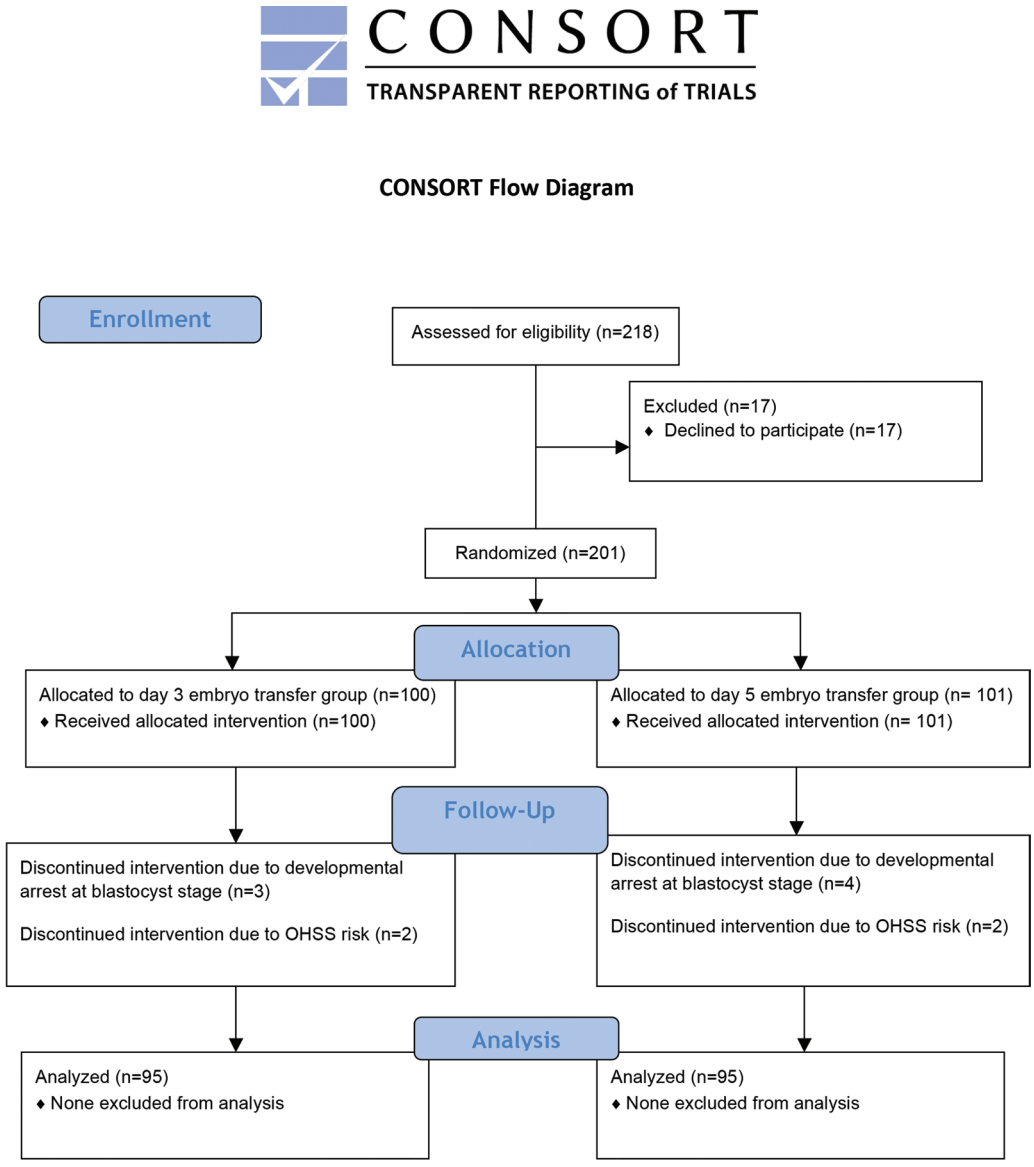

Figure 1. CONSORT flow diagram

OHSS: Ovarian hyperstimulation syndrome

G1.2 and G2.2 media (Scandinavian IVF Sciences, Gothenburg, Scandinavia) on day 1 and day 3, respectively. Standard culture medium was compared with sequential media because the use of G1.2 medium was not recommended for day 3 transfer. The number of blastomeres, the degree of fragmentation, and evenness of blastomere size for each embryo were recorded on days 2 and 3 for day 3 transfers and continued to be monitored on days 4 and 5 for day 5 transfers. A classification system introduced by Veeck was used for embryo assessment on the third day of culture. The embryos were classified as: grade 1, embryos with even blastomeres and no cytoplasmic fragments; grade 2, embryos with even blastomeres and minor cytoplasmic fragments or blebs; grade 3, embryos with uneven blastomeres and no or few cytoplasmic fragments[11]. Blastocysts were graded according to the size of blastocele cavity as early, full or expanded; the inner cell mass; and trophectoderm layer distribution.

Embryo transfer

One or two top-grade embryos from both groups were transferred into the endometrial cavity on day 3 and 5. In case of developmental delay on day 5, one or two of the most advanced embryos were transferred into the uterus on day 5. Women aged ≥35 years and/or those who had two previous unsuccessful cycles had the right to two embryos being transferred. The luteal phase was supported by 50 mg intramuscular progesterone in oil once daily (Progestan®, Koçak Farma, İstanbul, Turkey) and estradiol, two 100 µg transdermal patches (Estraderm TTS®, Novartis Pharma AG, Basel, Sweden) with daily replacements. A pregnancy test was performed on the 12th day following ET.

Women with a positive HCG underwent a transabdominal ultrasound scan 15 days later. Clinical pregnancy was defined as the identification of an intrauterine embryo with heart beat.

Statistical Analysis

The sample size was computed at the beginning of the study using an online analyzer (http://clincalc.com/Stats/SampleSize.aspx). For an expected difference of 15% between the two groups (10% vs. 25%) with a 5% level of significance and a power of 80%, a total of 200 patients (100 patients per group) were needed. Collected data were analyzed using the Statistical Package for Social Sciences version 18.0 (SPSS IBM Software, Armonk, NY, USA). Kolmogorov-Smirnov test, Fisher's exact test, and the chi-square test were used for statistical analyses. Two-tailed p values <0.05 were regarded as statistically significant.

Results

Forty-nine women (25.8%) had no previous ICSI cycles, and 67 women (35.3%) had one previous ICSI cycle, and 74 women (38.9%) had at least two previous ICSI cycles.

Table 1 demonstrates the clinical characteristics of the day 3 and day 5 ETs. Day 3 and day 5 transfers were statistically similar with respect to the age of the women (p=0.107), duration of infertility (p=0.528), cause of infertility (p=0.850), number of collected oocytes (p=0.119), number of metaphase II oocytes (p=0.178), number of fertilized oocytes (p=0.092), and number of transferred embryos (p=0.556). When compared with day 3 transfers, the number of grade 1 embryos were significantly higher in day 5 transfers (p=0.001).

Table 2 displays the pregnancy outcomes of the day 3 and day 5 ETs. Day 3 and day 5 ETs had statistically similar implantation, clinical pregnancy, and live birth rates (p=0.779, p=0.771, and p=0.649, respectively). The miscarriage and perinatal death rates were also statistically similar in day 3 and day 5 ETs (p=0.551 and p=0.407, respectively). Day 3 and day 5 ETs resulted in statistically similar twinning rates (p=0.183). Additionally, the miscarriage, perinatal death, and live birth rates in singleton pregnancies conceived after day 3 and day 5 ETs were found statistically similar (p=0.276, p=0.083, and p=0.594, respectively).

SET and double ETs at day 3 had statistically similar pregnancy (39.0% vs. 58.3%; χ^2=3.367; p=0.067) and live birth rates (32.2% vs. 44.4%; χ^2=1.440; p=0.230). Single and double blastocysts transfers at day 5 also had statistically similar pregnancy (43.6% vs. 45.0%; χ^2=0.017; p=0.895), and live birth rates (36.4% vs. 32.5%; χ^2=0.152; p=0.696). SETs at day 3 and day 5 yielded statistically similar pregnancy (39.0% vs. 43.6%; χ^2=0.254; p=0.614), and live birth rates (32.2% vs.

Table 1. Clinical characteristics of the embryo transfers

	Overall (n=190)	Day 3 transfers (n=95)	Day 5 transfers (n=95)	p
Age (years)	29.9±4.3 (22-39)	29.4±4.2	30.4±4.3	0.107
Infertility duration (years)	8.0±4.2 (1-16)	7.8±3.4	8.1±4.8	0.528
Infertility cause				0.850
Polycystic ovaries	112 (59.0%)	59 (62.1%)	53 (55.8%)	(χ^2=0.799)
Male factor	58 (30.5%)	27 (28.4%)	31 (32.6%)	
Tubal factor	11 (5.8%)	5 (5.3%)	6 (6.3%)	
Endometriosis	9 (4.7%)	4 (4.2%)	5 (5.3%)	
Collected oocytes per cycle	13.9±5.2 (4-25)	13.3±5.5	14.5±4.9	0.119
Metaphase II oocytes per cycle	8.4±3.3 (3-14)	8.1±3.4	8.7±3.2	0.178
Fertilized oocytes per cycle	6.6±3.2 (2-12)	6.2±3.0	7.0±3.5	0.092
Transferred embryos per cycle	1.4±0.5 (1-2)	1.4±0.6	1.4±0.4	0.556
Embryo grade				0.001*
Grade 1	25 (13.2%)	5 (5.3%)	20 (21.1%)	(χ^2=17.642)
Grade 2	112 (58.9%)	53 (55.8%)	59 (62.1%)	
Grade 3	53 (27.9%)	37 (38.9%)	16 (16.8%)	
Transferred embryos				0.001*
2-cell	13 (6.8%)	13 (6.8%)	0 (0.0%)	(χ^2=190.000)
3-cell embryos	18 (9.5%)	18 (9.5%)	0 (0.0%)	
4-cell embryos	64 (33.7%)	64 (33.7%)	0 (0.0%)	
>4-cell embryos	95 (51.0%)	0 (0.0%)	95 (100.0%)	
Blastocyst grade				
Early			45 (47.4%)	
Full			19 (20.0%)	
Expanded			31 (32.6%	

*p<0.05 was accepted as statistically significant

Table 2. Pregnancy outcomes of the embryo transfers

	Overall (n=190)	Day 3 transfers (n=95)	Day 5 transfers (n=95)	p
Implantation rate	88 (46.3%)	45 (47.4%)	43 (45.3%)	0.779 (χ^2=0.107)
Clinical pregnancies	86 (45.2%)	44 (46.3%)	42 (44.2%)	0.771 (χ^2=0.085)
Singleton	74 (38.9%)	40 (42.1%)	34 (35.8%)	0.183 (χ^2=1.774)
Twins	12 (6.3%)	4 (4.2%)	8 (8.4%)	
Miscarriages	12 (6.3%)	7 (7.4%)	5 (5.3%)	0.551 (χ^2=0.356)
Singleton	7 (3.7%)	4 (4.2%)	3 (3.2%)	0.276 (χ^2=1.185)
Twins	5 (2.6%)	3 (3.2%)	2 (2.1%)	
Perinatal deaths	6 (3.2%)	2 (2.1%)	4 (4.2%)	0.407 (χ^2=0.688)
Singleton	4 (2.1%)	2 (2.1%)	2 (2.1%)	0.083 (χ^2=3.000)
Twins	2 (1.1%)	0 (0.0%)	2 (2.1%)	
Live births	68 (35.8%)	35 (36.8%)	33 (34.7%)	0.649 (χ^2=0.207)
Singleton	63 (33.2%)	34 (35.8%)	29 (30.5%)	0.594 (χ^2=0.284)
Twins	5 (2.6%)	1 (1.0%)	4 (4.2%)	

p<0.05 was accepted as statistically significant

36.4%; χ^2=0.219; p=0.640). Double ETs at day 3 and day 5 also resulted in statistically similar pregnancy (58.3% vs. 45.0%; χ^2=1.348; p=0.246), and live birth rates (44.4% vs. 32.5%; χ^2=1.146; p=0.284).

Discussion

SET has been instituted as a governmental policy in many European countries including Turkey. The reason for adopting such a policy is to avoid multiple pregnancies. Thus, the IVF centers dealing with SET have focused on identifying top quality embryos and designating the optimal time for its transfer. In other words, an embryo with the highest chance of implantation should be selected to achieve satisfactory pregnancy rates[1-4].

It has been reported that morphologic assessment and grading of day 2 or 3 embryos have limited predictive value for further embryonic development[12-14]. Related studies have also indicated that recruitment at the blastocyst stage yields better results than selection at day 3, which merely depends on the morphologic evaluation of embryos. These studies also claim that pregnancy rates of up to 50% can be acquired by the transfer of blastocysts when compared with embryo transfer at the cleavage stage[15-21]. Similarly, it has been shown that the risk of aneuploidy was significantly lower in day 5 embryos than in day 3 embryos[22]. However, it has not been conclusively specified that blastocyst transfers have better perinatal outcomes than day 3 ETs[23]. In fact, a prospective observational study showed that about 50% of embryos conceived in 224 IVF/ICSI cycles were exposed to developmental arrest at the blastocyst stage[24].

The rationale of blastocyst transfer is based on increasing the probability of obtaining advanced embryos with the highest chance for survival, i.e., implantation. The prolongation of embryo culture to day 5 requires a relatively high number of top quality blastocysts. Good quality cleavage-stage embryos increases the likelihood of good quality blastocyst embryos. Therefore, it would be prudent to expect no advantage if only a few good quality blastocysts exist in the culture[20,25,26].

The transfer of two blastocysts at day 5 was more favorable than two embryos at day 3 in a cohort of 164 infertile women aged <37 years in a randomized controlled trial. In that study, transfers at blastocyst stage resulted in significantly higher pregnancy (51.3% vs. 27.4%) and live birth (47.5% vs. 27.4%) rates than the transfers at the cleavage stage. Yet, the twinning rate was statistically similar for the day 3 and day 5 transfers (36.8% vs. 30.4%)[19]. In accordance with this result, a prospective randomized study indicated that single blastocyst transfers had significantly higher pregnancy rate (23.7% vs. 35.6%) than the SETs in a cohort of 227 infertile women aged <36 years and who were on their first or second IVF trials[26].

Contrary to the study above, another prospective randomized study reported that day 3 and day 5 transfers yielded statistically similar overall implantation (21% vs. 23%), pregnancy (39% vs. 39%) and twinning (11.9% vs. 15%) rates in a cohort of 201 infertile women[27]. Likewise, day 3 and day 5 transfers resulted with statistically similar implantation (47.4% vs. 45.3%), clinical pregnancy (46.3% vs. 44.2%), live birth (36.8% vs. 34.7%), and twinning (4.2% vs. 8.4%) rates in this study.

A review of related literature pointed out that day 5 ET with expanded blastocysts had a significantly higher implantation rate than non-expanding or non-cavitating embryos and, thus, single blastocyst transfer might be more successful than SET[26,27]. For instance, about 60% of women who received single blastocyst transfers had at least one excellent quality blastocyst and the pregnancy rate was 40.8% for these patients in a Belgian study[26]. Another study conducted in Saudi Arabia demonstrated that the success of day 5 transfers was a continuum of the number of good quality cleavage stage embryos and the availability of at least one blastocyst for

transfer[27]. Correspondingly, the blastocyst transfers at day 5 could end up with higher implantation rates than early-stage ETs if at least one blastocyst is available for day 5 transfers.

The present study failed to detect statistically significant differences in implantation, clinical pregnancy, and live birth rates of day 3 and day 5 transfers. This inability can be attributed to the relatively small cohort size and relative heterogeneity in study population. The variations in clinical characteristics of the patients (i.e., age, cause of infertility, duration of infertility) might have caused such discrepancy.

Study Limitations

Relatively small sample size for statistical analysis is the limitation of the study.

Conclusion

In conclusion, the efficacy of blastocyst stage ET is not inferior to cleavage stage ET. Performing blastocyst transfer may have benefits because it is associated with satisfactory pregnancy rates and the assessment of embryo morphology at day 3 has limited predictive value for subsequent developmental process. The maintenance of embryo culture until day 5 may be a more sensible approach for the correct identification of best quality embryos with the highest probability of success for implantation.

The findings of the present study imply that blastocyst transfer at day 5 can be adopted as a reasonable approach in Turkish women who are to undergo elective SET or double ET. In order to maximize the probability of conceiving a pregnancy in an elective blastocyst transfer, this practice should be offered for ICSI cycles, in which several good quality embryos are obtained at day 3. However, patients who are to receive blastocyst transfer at day 5 should be informed that there is a greater risk of cycle cancellation because the embryo culture is extended to the blastocyst stage. Further research is warranted to determine the efficacy of blastocyst transfer at day 5 in Turkish women undergoing elective SET or double ET.

Ethics

Ethics Committee Approval: The study was approved by the Afyon Kocatepe University Local Ethics Committee (approval number: 149.01.2016).

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Role of osteocalcin, tumor necrosis factor-alpha and adiponectin in polycystic ovary syndrome patients with insulin resistance

Polikistik over sendromlu hastalarda insülin direncinde osteokalsin, tümör nekroz faktörü-alfa ve adiponektinin rolü

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Abstract

Objective: Insulin resistance (IR) seems to be the main pathogenic factor in polycystic ovary syndrome (PCOS). Adiponectin and tumor necrosis factor-alpha (TNF-α) are important in IR. The aim of this study was to evaluate the correlations of osteocalcin, adiponectin, and TNF-α with IR in PCOS.
Materials and Methods: A total of 60 women were divided into two groups. The first group constituted 44 patients with PCOS and the control group comprised 16 healthy women. Osteocalcin, adiponectin, TNF-α levels, body mass index (BMI), and IR in the fasting state were assessed and correlations of these parameters were evaluated.
Results: Homeostasis model assessment (HOMA)-IR, adiponectin, osteocalcin, and androstenedione levels were significantly increased in the PCOS group. A moderate positive correlation between BMI and HOMA-IR, a moderate negative correlation between TNF-α and osteocalcin, and a mild negative correlation between adiponectin and BMI were detected in PCOS.
Conclusion: Osteocalcin may have impact on adiponectin, TNF-α, and IR levels in PCOS. Different osteocalcin levels in patients with PCOS may be responsible for explaining PCOS heterogeneity.
Keywords: Polycystic ovary syndrome, insulin resistance, osteocalcin, adiponectin, tumor necrosis factor-alpha

Öz

Amaç: Polikistik over sendromunun (PKOS) temelinde yatan en önemli mekanizma insülin direncidir (IR). Bu çalışmada PKOS'li hastalarda kan osteokalsin düzeyinin; IR, adiponektin, tümör nekroz faktörü-alfa (TNF-α) ve bazı hormonal parametrelerle olan ilişkisini değerlendirmeyi amaçladık.
Gereç ve Yöntemler: PKOS tanısına uyan 44 hasta ve hiçbir rahatsızlığı olamayan 16 sağlıklı kontrol grubundan olusan toplam 60 hasta ile yapıldı. Bu hastaların kan osteokalsin, adiponektin, TNF-α, vücut kitle indeksi (VKİ) ve IR düzeyleri ölçülerek bu parametreler arasındaki ilişki incelendi.
Bulgular: PKOS grubunda homeostaz model değerlendirmesi (HOMA)-IR, adiponektin, osteokalsin ve androstenedion düzeyleri anlamlı olarak yüksek bulundu.VKİ ve HOMA-IR arasında pozitif ilişki bulunurken TNF-α ve osteokalsin, adiponektin ve VKİ arasında negatif ilişki saptandı.
Sonuç: PKOS'li hastalarda kan osteokalsin düzeyinin adiponektin, TNF-α, ve IR üzerine etkisi bulunmaktadır. Saptanan farklı osteokalsin düzeyleri PKOS'li hastalarda heterojeniteyi açıklamakta faydalı olabilir.
Anahtar Kelimeler: Polikistik over sendromu, insülin direnci, osteokalsin, adiponektin, tümör nekroz faktörü-alfa

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PRECIS: Bone is recognized as an endocrine organ. Osteocalcin seems to play a key role in the heterogeneity of polycystic ovary syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is a common and heterogeneous disease characterized by anovulation, hyperandrogenism, and/or polycystic ovaries[1,2]. Therefore, an important consideration is whether such adipocytokines as adiponectin, a potential mediator of insulin resistance (IR), are also implicated in the pathogenesis of PCOS[3]. Levels of adiponectin, an abundant adipocyte-derived cytokine, are strongly correlated with measures of IR[4,5]. Gonzalez et al.[6] illustrated that hyperglycemia caused an increase in reactive oxygen species (ROS) generation from peripheral blood mononuclear cells (MNC). ROS-induced oxidative stress is a known activator of nuclear factor B, a proinflammatory transcription factor that promotes tumor necrosis factor (TNF) gene transcription[7]. TNF was established as a mediator of IR by Hotamisligil et al.[8]. Thus, increased TNF release from MNC in response to hyperglycemia may be an underlying mechanism for IR in PCOS.

Previous animal studies showed that osteocalcin stimulated the expression of insulin in islets and of adiponectin in adipocytes with increased insulin secretion and sensitivity[9]. Reduced osteocalcin levels have been claimed to be associated with diabetes mellitus (DM) development[10]. We aimed to evaluate the correlations of blood osteocalcin, adiponectin, and TNF-α levels with IR in PCOS. Additionally, we evaluated the relationship of these with some hormonal parameters.

Materials and Methods

A total of 60 women including 44 patients with PCOS and 16 healthy women (control group) were studied at Erciyes University Gynecology Clinic. The diagnosis of PCOS was based on the established guidelines by the PCOS Consensus Workshop Group[1]. Ultrasonographic diagnosis of polycystic ovaries was based on the presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or increased ovarian volume >10 mL on pelvic or vaginal ultrasound examination. Oligomenorrhea was defined as the absence of menstruation for 35 days or more and amenorrhea was defined as the absence of menstruation for 3 months or more[1].

All women were examined both clinically and gynecologically including ultrasonography. Body weight, height, and body mass index (BMI) were recorded. The BMI was calculated as weight/(height)^2 in kilograms per square meter. The study and control groups were weight matched. Patients with congenital adrenal hyperplasia, androgen-producing tumors, adrenal dysfunction, Cushing's syndrome, hyperprolactinemia, DM, liver, kidney, heart, and thyroid diseases were excluded from the study. None of the women in study or control group had taken medications known to effect plasma sex steroids for ≥6 months before the study and none of the volunteers was a cigarette smoker. All the women agreed to participate in the present study. The study was approved by the Ethics Committee of Erciyes University Hospital (approval number: 2011-369) and written informed consent was obtained from each woman. Moreover, we obtained an Australian-New Zealand clinical trials registry number: 12613001132730.

Fasting state venous blood was collected from the subjects during the midfollicular phase of the menstrual cycle between 08:00 am and 09:00 am. Glucose levels were measured three days after the normal diet and normal daily activity using the oxidase method with Konelab 60-i auto-analyzers (Thermo Clinical Labsystem, Finland). IR in the fasting state was assessed by using homeostasis model assessment (HOMA) and was calculated with the following formula: fasting plasma glucose (mmol/L) × fasting serum insulin (μU/mL) divided by 22.5, as described by Matthews et al.[11]. Hormonal analyses included: thyroid stimulating hormone, dehydroepiandrosterone sulfate (DHEAS), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), 17-hydroxyprogesterone (17-OHP), androstenedione (A), free testosterone (fT), total testosterone (tT), insulin, and sex hormone binding globulin (SHBG) levels. tT and fT (Biosource, Nivelles, Belgium), 17-OHP (DSL-3500, Texas, USA), DHEAS (Immunotech, Marseille, France), A (DSL-3800, Texas, USA) were measured using an immunoradiometric assay and its commercial kit, serum SHBG (Zentech, Angleur, Belgium), insulin (Biosource, Nivelles, Belgium), LH, FSH, P, PRL (ACS:180, Bayer, Germany) were measured using chemiluminescence and a commercial kit. After centrifugation, blood serum was stored at -70 °C until assayed. Adiponectin (Adiponectin kit, Assaypro, UK), TNF-α (TNF-α Invitrogen 96 Tests, UK) and osteocalcin (Gla-type osteocalcin in vitro enzyme immunoassay kit, Takara Bio Inc., UK) were measured using an enzyme-linked immunosorbent assay.

The intra and inter-assay precision coefficients of variation were 2.8% and 4.6% for FSH, 5% and 6.2% for LH, 9.9% and 11.8% for E2, 4.4% and 4.8% for testosterone, 4.3% and 7.8% for fT, 11% and 2.8% and 7% for A, 6.3% and 9.9% for DHEAS, 9.5% and 10.8% for 17-OHP, 5.2% and 5.8% for SHBG, and 1.6% and 6.1% for insulin, respectively. All results are expressed as means ± standard deviation.

Statistical Analysis

The Shapiro-Wilk test was used to check the normality assumption of the data. Independent samples t-test and Mann-Whitney U tests were used to compare the differences of variables between the groups. Pearson and Spearman analysis were used to examine correlations, and a scatterplot matrix was also produced to display pairwise relationships between

variables. To identify independent risk factors of PCOS, univariate and multivariate logistic regression analysis was used and odds ratios were calculated with their 95% confidence intervals. Statistically significant variables in univariate analysis were included in the multivariate logistic model and backward stepwise selection was performed at a stringency level of $p<0.10$ to determine the independent risk factors of PCOS. Two-sided p values <0.05 were considered statistically significant.

Results

The study and control groups were weight matched. Hormone levels and baseline characteristics of the groups are illustrated in Table 1.

The level of A was significantly high in the PCOS group. There was no statistically significant difference between the groups for age, BMI, DHEAS, FSH, SHBG, LH, fT, tT, and E2.

High levels of HOMA-IR, adiponectin, and osteocalcin were detected in the PCOS group. There was no significant difference between the two groups for TNF-α (Table 1). The cut-off value of HOMA-IR was accepted as 2.5[12,13].

We detected a strong positive correlation between adiponectin and osteocalcin in the control group. There was positive correlation between osteocalcin and BMI in addition to a negative correlation between osteocalcin and TNF-α in the PCOS group. We found a moderate positive correlation between BMI and HOMA-IR, a moderate negative correlation between TNF-α and osteocalcin, and a mild negative correlation between adiponectin and BMI (Table 2, Figure 1).

Discussion

Many of the symptoms appear to be quite heterogeneous, with marked differences in their prevalence and intensity among different groups of women with PCOS. IR was significantly high in the PCOS group. Some studies showed IR only in obese women with PCOS and others demonstrated IR in lean patients with PCOS. Of importance, the studies that failed to demonstrate IR in lean women with PCOS did, however, demonstrate elevated basal insulin levels compared with weight-matched controls without PCOS[14].

The groups in our study were weight matched; therefore, the effect of adipose tissue on TNF-α and adiponectin was eliminated. We found higher levels of adiponectin in PCOS; however, some authors suggested that women with PCOS had lower adiponectin levels[15]. Conversely, an increment in plasma adiponectin was obtained by Frystyk et al.[16] in type 1 DM. One way to interpret the present findings is to conclude that high adiponectin levels may be an early predictor of DM development. Unfortunately, 52.7% of patients in the PCOS group had IR. However, the finding can also be interpreted in the opposite way, as elevated adiponectin levels could represent a beneficial compensatory mechanism. Several markers of inflammation are increased in PCOS, which suggests that it is

Table 1. Hormonal levels and baseline characteristics of groups

Variables	Between-group comparisons			Logistic regression analyses	
	PCOS group (n=44)	Control group (n=16)	p	Univariate OR (95% CI)	Multivariate OR (95% CI)
Age (years)	21.50 (20.00-24.00)	22.50 (19.50-24.75)	0.608	0.96 (0.81-1.14)	-
BMI (kg/m^2)	22.50 (20.25-26.00)	21.00 (20.00-23.00)	0.072	1.27 (0.98-1.65)	-
tT (pg/mL)	80.50 (49.75-113.25)	72.00 (61.25-93.00)	0.77	1.01 (0.99-1.02)	-
fT (pg/mL)	2.64±0.83	2.22±1.24	0.217	1.64 (1.60-3.14)	-
A (ng/mL)	3.05 (2.27-4.12)	2.24 (2.00-2.98)	0.026	1.91 (1.03-3.54)	-
DHEAS (ng/mL)	2182.34±125.44	2334.37±768.27	0.652	1.00 (0.99-1.01)	-
SHBG (nmoL/mL)	47.00 (26.50-95.50)	49.50 (41.25-85.75)	0.477	1.00 (0.99-1.01)	-
FSH (pg/mL)	5.00 (4.00-4.00)	5.30 (4.45-7.75)	0.317	0.76 (0.54-1.08)	-
LH (pg/mL)	5.30 (4.42-9.00)	8.00 (5.30-11.75)	0.083	0.96 (0.87-1.05)	-
E2 (pg/mL)	77.00 (50.00-95.00)	73.00 (57.00-105.00)	0.553	1.00 (0.99-1.01)	-
HOMA-IR	2.35 (1.50-3.20)	1.35 (1.08-2.37)	0.01	2.25 (1.10-4.57)	2.70 (1.21-6.01)
Adiponectin (µg/mL)	64.67 (61.08-68.18)	60.52 (59.16-62.30)	0.011	1.22 (1.03-1.44)	-
TNF-α	33.02 (12.29-86.05)	15.46 (13.13-29.08)	0.288	1.00 (0.99-1.01)	-
Osteocalcin (ng/mL)	1.96 (1.45-2.36)	1.01 (0.79-1.39)	<0.001	15.39 (3.25-72.99)	21.61 (3.73-125.27)

PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, tT: Total testosterone, fT: Free testosterone, A: Androstenedione, DHEAS: Dehydroepiandrosterone sulfate, SHBG: Sex hormone binding globulin, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, HOMA-IR: Homeostasis model assessment-insulin resistance, TNF-α: Tumor necrosis factor-alpha

Table 2. Correlation of osteocalcin level with hormonal levels, age, body mass index, homeostasis model assessment-insulin resistance, tumor necrosis factor-alpha, and adiponectin for both groups

Variables	Control group Osteocalcin (p)	PCOS group Osteocalcin (p)
Age	0.068 (0.802)	-0.333 (0.027)
BMI	0.040 (0.882)	0.638 (0.027)
tT	0.066 (0.80)	0.175 (0.257)
fT	-0.001 (0.996)	0.055 (0.725)
A	0.234 (0.383)	-0.139 (0.367)
SHBG	-0.341 (0.196)	-0.041 (0.793)
DHEAS	0.200 (0.458)	0.022 (0.889)
FSH	-0.086 (0.752)	-0.054 (0.726)
LH	0.021 (0.940)	0.154 (0.318)
E2	0.337 (0.201)	0.054 (0.727)
Adiponectin	0.671 (0.004)	-0.061 (0.695)
TNF-α	0.344 (0.192)	-0.338 (0.025)
HOMA-IR	0.091 (0.736)	-0.155 (0.314)

PCOS: Polycystic ovary syndrome, BMI: Body mass index, tT: Total testosterone, fT: Free testosterone, A: Androstenedione, SHBG: Sex hormone binding globulin, DHEAS: Dehydroepiandrosterone sulfate, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TNF-α: Tumor necrosis factor-alpha, HOMA-IR: Homeostasis model assessment-insulin resistance

a state of chronic low grade inflammation. Keeping in mind the anti-inflammatory and anti-DM properties of adiponectin, one could hypothesize that increased adiponectin levels serve to protect patients at high risk of the harmful actions of pro-inflammatory and DM agents.

Increased levels of TNF-α were detected in PCOS; however, there was no statistical difference between the groups. Although Vural et al.[17] could not illustrate higher TNF-α levels in PCOS, Xiong et al.[18] suggested that patients with PCOS showed significantly higher serum TNF-α levels. The pathogenic impact of TNF-α in IR is underscored by the effect of the functional polymorphisms in the promoter regions of TNF-α, with different transcription rates[19], or this situation may be related to the balance of anti-inflammatory and inflammatory agents that are secreted by bone and adipose tissue in PCOS.

More recently, evidence from animal studies suggested that the skeleton may exert an endocrine regulation of glucose metabolism. Lee et al.[20] showed that mice lacking the gene that encodes osteocalcin had an abnormal amount of visceral fat and exhibited glucose intolerance, IR, and impaired insulin secretion compared with wild-type mice. Adami et al.[21] could not illustrate a significant difference between a PCOS group and

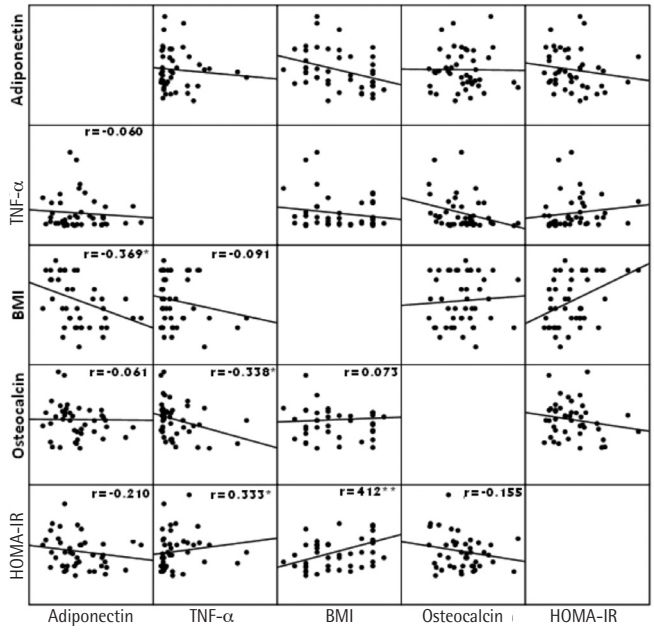

Figure 1. A scatterplot matrix displaying the relationship among body mass index, homeostasis model assessment-insulin resistance, tumor necrosis factor-alpha, adiponectin, and osteocalcin variables

*p<0.05, **p<0.01

HOMA-IR: Homeostasis model assessment-insulin resistance, BMI: Body mass index, TNF-α: Tumor necrosis factor-alpha

control group for osteocalcin, but they found normal androgen levels in their PCOS group; additionally, they did not examine patients for IR. In our study, osteocalcin was significantly increased in PCOS; moreover, there was a negative correlation between osteocalcin and TNF-α. Our study is in agreement with Diamanti-Kandarakis et al.[22] who illustrated higher osteocalcin levels in PCOS.

There was no correlation between serum adiponectin and HOMA-IR. There was a moderate negative correlation between osteocalcin and TNF-α, in addition to a moderate positive correlation between BMI and HOMA-IR in PCOS. Adiponectin secretion is strongly related with IR rather than obesity, and a previous animal study showed that osteocalcin stimulated the expression of insulin in islets and of adiponectin in adipocytes with increased insulin secretion[23]. Perhaps increased osteocalcin levels contribute to high HOMA-IR by increased insulin secretion. Our groups were weight matched and the source of adiponectin was adipose tissue. This situation may explain why we did not detect a correlation between serum adiponectin levels and HOMA-IR.

TNF-α can be released from MNCs and hyperglycemia causes an increase in ROS generation from MNCs. Osteocalcin is defined in the literature with antidiabetic and anti-inflammatory properties, thus a plausible explanation of these events is that increased osteocalcin levels lead to a decrease in TNF-α.

Study Limitations

Due to the relatively small sample size, our results display only weight-matched controls in PCOS. There is a need for further, larger scale studies including interactions between other genetic and environmental factors and the development of PCOS.

Conclusion

Osteocalcin levels may have impact on adiponectin, TNF-α, and IR in PCOS. Therefore, osteocalcin may be responsible for PCOS heterogeneity.

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Ethics

Ethics Committee Approval: The study was approved by the Erciyes University Local Ethics Committee (approval number: 2011-369).
Informed Consent: Consent form was filled out by all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Effect of pigtail catheter application on obstetric outcomes in *in vitro* fertilization/intracytoplasmic sperm injection pregnancies following hyperstimulation syndrome

Ovaryan hiperstimülasyon sendromu tedavisinde pigtail kateter uygulamasının in vitro fertilizasyon/intrasitoplazmik sperm enjeksiyonu gebeliklerinde obstetrik sonuçlara etkisi

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Abstract

Objective: To evaluate the effects of percutaneous pigtail catheter drainage on the outcomes of intracytoplasmic sperm injection (ICSI) pregnancies following moderate or severe ovarian hyperstimulation syndrome (OHSS).
Materials and Methods: This retrospective study included 189 patients hospitalized for OHSS following ICSI treatment in a tertiary *in vitro* fertilization unit between 2006 and 2014. Pigtail catheters were applied in 63 patients; the other 126 patients did not need that treatment. The obstetric reports of 173 patients could be accessed and were examined to investigate the pregnancy outcomes of those with and without catheters.
Results: No complications such as infection or vascular or intra-abdominal organ trauma were observed related to the pigtail application. There were no differences in abortus, preterm labor, gestational diabetes mellitus, and preeclampsia ratio between the pigtail and control groups (p>0.05). The rate of readmission to hospital for OHSS was lower in the pigtail group than in the control group although not statistically significant (p=0.08).
Conclusion: Pigtail application is a safe and effective method for draining ascites in patients with OHSS after ICSI treatment. The use of pigtail catheters had no adverse effects on the perinatal outcomes of patients hospitalized with OHSS who became pregnant after ICSI treatment. In addition, the percutaneous drainage of ascites via a pigtail catheter helped prevent the readmission of patients with moderate or severe OHSS.
Keywords: Ovarian hyperstimulation syndrome, ascites, pigtail catheter, *in vitro* fertilization

Öz

Amaç: Orta ve ciddi ovaryan hiperstimülasyon sendromunda (OHSS) kullanılan perkütan pigtail kateter uygulamasının, intrasitoplazmik sperm enjeksiyonu (ICSI) uygulaması sonucu oluşan gebelik sonuçlarına etkisini araştırmayı amaçladık.
Gereç ve Yöntemler: Retrospektif olan bu çalışma 2006-2014 yılları arasında tersiyer bir *in vitro* fertilizasyon ünitesinde uygulanan ICSI sonucunda gelişen OHSS nedeniyle yatarak tedavi edilen 189 hastayı içermektedir. Bu hastalardan 63 hastaya pigtail kateteri uygulanmış, 126 hastaya uygulamaya gerek kalmamıştır. Yüz yetmiş üç hastanın obstetrik sonuçlarına ulaşılabilmiş, bu hastalarda kateter uygulanan ile uygulanmayan arasında obstetrik sonuçlar karşılaştırılmıştır.
Bulgular: Pigtail kateter uygulamasına bağlı hiçbir hastada enfeksiyon, vasküler veya intraabdominal organ yaralanması gibi komplikasyonlar izlenmemiştir. Pigtail kateter uygulanan ve uygulanmayan hastalarda düşük, erken doğum, gestasyonel diabetes mellitus ve preeklampsi oranlarında fark izlenmemiştir (p>0,05). Pigtail kateter uygulanan hasta grubunda ise hastanın OHSS nedeniyle hastaneye ikinci yatış oranı istatistiksel olarak anlamlı olmasa da daha düşük izlenmiştir (p=0,08).
Sonuç: ICSI tedavisi nedeniyle gelişen OHSS'de batında distansiyon yaratan asitin direnajında pigtail kateter kullanımı güvenli ve etkindir. Perkütan direnaj kateteri olan pigtail kateterinin, uygulanan hasta grubunda uygulanmayanlara göre perinatal sonuçlarında fark izlenmemiştir. Buna ek olarak, OHSS semptomlarını azaltması amacıyla uygulanan pigtail kateteri, hastaların aynı şikayetlerle hastaneye mükerrer yatışlarında azalma sağlamıştır.
Anahtar Kelimeler: Ovaryan hiperstimülasyon sendromu, asit, pigtail kateter, *in vitro* fertilizasyon

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PRECIS: Pigtail application is a safe and effective in draining ascites in patients with ovarian hyperstimulation syndrome after intracytoplasmic sperm injection treatment and had no adverse effects on perinatal outcomes.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a serious and sometimes life-threatening complication of *in vitro* fertilization (IVF) treatment. Although various preventive measures are taken, the incidence of OHSS is still around 2%[1], and management plays an important role in this potentially lethal complication. There are no OHSS-free clinics, although there are preventive measures such as freeze-all protocols and lower ovarian stimulation IVF protocols.

Mild-to-severe forms of clinical findings such as leukocytosis, hemoconcentration, hypovolemia, edema, ascites, hydrothorax, pericardial effusion, thromboembolic events, oligo or anuria, acute respiratory distress syndrome, and multiple organ failure can be seen due to extravasation of intravascular protein-rich fluid to the third spaces. Ascites form as a result of the underlying pathophysiology and aggravate the course of the disease. In the management of OHSS, it is necessary to eliminate the ascites to alleviate symptoms, which include abdominal discomfort and dyspnea. Abdominal paracentesis may need to be performed several times, and it is usually done blindly. An easier and safer method of draining ascites is to use a pigtail catheter, which is inserted easily into the abdomen percutaneously under local anesthesia and guided using ultrasonography[2]. Pigtail catheters have proven to be safe for draining ascites fluid. However, it is not clear whether this invasive procedure creates any procedure-related risk for pregnant patients such as bleeding, infection, trauma to neighbouring intraabdominal organs or preterm delivery.

To the best of our knowledge, there are no data comparing the obstetric outcomes of patients with pigtail catheters and controls. In our study, therefore, we evaluated the obstetric outcomes of patients who experienced an IVF/intracytoplasmic sperm injection (IVF/ICSI) pregnancy following OHSS, and compared patients with and without pigtail catheters.

Materials and Methods

Patient population

Between May 2006 and September 2014, a total of 9289 consecutive IVF/ICSI cycles were performed at our University Hospital, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and IVF Unit. One hundred and eighty-nine number of patients who were hospitalized due to moderate or severe OHSS with ascites were included in the study. Those whose obstetric data could not be obtained were excluded. We could be able to access the obstetric outcomes of 173 number of patients treated with or without pigtail catheters in two separate groups. Abortus ratios, preterm labor ratios, preterm premature rupture of membrane (PPROM) ratios, and other pregnancy outcomes such as preeclampsia, intrauterine growth retardation (IUGR), gestational diabetes mellitus (GDM), and birth weights and birth weeks of gestations were analyzed between the two groups. The study was approved by the ethics committee of the university. This is a retrospective study; therefore, informed consent was not required from the patients.

Ovarian hyperstimulation syndrome treatment and follow-up

The classification of OHSS was defined according to the criteria of Golan et al.[3] and Navot et al.[4]. Moderate OHSS was defined as abdominal distention and discomfort, nausea, vomiting, diarrhea, enlarged ovaries 5-12 cm in diameter, and presence of ascites. Severe OHSS was defined as massive ascites, hydrothorax, breathing difficulties, hematocrit >45%, leukocytes >15.000, creatinine clearance ≤50 mL/min, oliguria, and liver dysfunction.

In our clinic, hospitalization is recommended for patients with moderate and severe OHSS to facilitate close monitoring. During follow-up, patients with OHSS are treated with bed rest, and the following measurements are taken every day: body weight, waist circumference, vital signs every four hours, urine output, and intravenous or oral fluid intake. Total daily fluid intake is limited to 3000 mL/day. If the patient cannot tolerate oral fluid intake, parenteral replacement is administered. Hydroxyethyl starch (HES) solution (6% HES 130/0.4 in 0.9% sodium chloride injection; Voluven®, Germany) is added to the parenteral solution to inhibit the shifting of intravenous fluid to the extracellular plain. If abdominal distention and discomfort are too high and oliguria is present, a pigtail catheter (8-0 French Flexima Drainage Catheter®; Boston Scientific, USA) is applied. During follow-up, if the ascites does not regress, a human albumin (human albumin 20%, 100 mL; Octapharma, Austria) infusion is given to patients who have decreased serum albumin levels. To prevent deep venous thrombosis, it is suggested that patients wear venous support stockings, and low-molecular-weight heparin (5000 units per day) is ordered. When morning hematocrit levels are ≤38% or the patients' symptoms are alleviated, discharge from the hospital is recommended.

The following patient data were recorded: age; infertility duration; IVF indication; IVF protocol; total gonadotropin doses used in IVF; estradiol level on human chorionic gonadotropin (hCG) day; number of follicles collected on oocyte pickup (OPU) day; total HES or albumin solution infused during treatment; hematocrit, leukocyte, platelet, and electrolyte counts on the days of admission to and discharge from hospital; length of stay in hospital; readmission to hospital, if necessary; clinical pregnancy and live birth rate; singleton, twin, or triplet pregnancy; and infant birth weight.

During the obstetric follow-up, the following tests were administered to diagnosis GDM. First, a 50-g oral glucose challenge test was performed between 24-28 weeks of gestation; if the first-hour glucose level was >140 mg/dL, a 100-g oral glucose tolerance test (OGTT) was performed. In the case of familial

history of diabetes mellitus or history of GDM during previous pregnancy, OGTT is suggested just after the first visit. Fasting, first-, second-, and third-hour glucose values greater than 95, 180, 155, and 140 mg/dL, respectively, were diagnosed as GDM. Preeclampsia was defined as blood pressure ≥140/90 mmHg measured at two different times and ≥300 mg proteinuria in 24-hour collected urine. Preterm labor was defined as the beginning of uterine contractions prior to 37 weeks' gestation. PPROM was defined as rupture of the amniotic membrane prior to 37 weeks' gestation. Intrauterine growth restriction was defined as weight below the tenth percentile for gestational age, which was measured sonographically. Miscarriage was categorized as either early (before 12 weeks' gestation), or late (after the 12th week). A chemical abortus was diagnosed in the presence of elevated hCG level in the patient's serum without demonstrating a gestational sac in the transvaginal sonographic examination.

Statistical Analysis

In the statistical analyses, demographic data of the patients are given as observational values, percentage rates, mean values, and standard deviation values. The chi-square test was used in the correlation analysis of categorical data while comparing the groups, and the Mann-Whitney U test was used in the paired comparison of continuous data, which generally did not comply with normal distribution. In addition, observation numbers obtained on the basis of catheter status were compared by applying the method of comparison of two rates. The data were analyzed using SPSS for Windows version 18.0 (SPSS, Inc., USA).

Results

Between 2006 and 2014, 9289 patients underwent IVF/ICSI procedures at the University Hospital, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and IVF Unit. Of those patients, 189 (2%) were hospitalized due to severe or moderate OHSS.

Of the 189 patients hospitalized due to OHSS, 33.3% (n=63) required a pigtail catheter and 66.6% (n=126) did not need catheterization for abdominal ascites drainage. We found no differences between the two groups in terms of age; total gonadotropin dose given during IVF treatment; estradiol blood level on the day of hCG; oocyte number after OPU; duration of infertility; or hemoglobin, hematocrit, and platelet levels on the first and last days of hospitalization. With the exception of the last day, the catheterized patients had higher platelet levels. In addition, the length of hospitalization was longer (p<0.01) and the HES and human albumin solution levels used were higher (p<0.01) in the catheterized group (Table 1). The duration of

Table 1. Characteristics of the two groups

	Non-catheter group (n=126)	Catheter group (n=63)	p
Age (years)	29.8±4.6	29.3±4.2	0.47
Total dose of gonadotropins used in IVF (mIU/mL)	1731±709	1835±791	0.44
Estradiol levels on day of hCG (pg/mL)	2933±1535	3250±2209	0.33
Oocyte count obtained after OPU process (n)	21±9	22±10	0.53
Infertility duration (year)	6.3±4.3	6.7±4.2	0.55
Hb count on first day of hospitalization (g/dL)	14.3±1.8	14.7±1.5	0.12
Hct ratio on first day of hospitalization (%)	42.5±5.5	43.7±4.6	0.14
PLT count on first day of hospitalization (106/mL)	342±81	368±94	0.05
Hb count on last day of hospitalization (g/dL)	11.7±1	12±1	0.32
Hct ratio on last day of hospitalization (%)	34.9±2.7	35±3	0.06
PLT count on last day of hospitalization (10^6/mL)	277±66	326±79	<0.01
Length of hospitalization (days)[a]	3.1±1.9	7.7±3.6	<0.01
Total HES solution (500 mL)[a]	1.34±1.4	3.7±3.7	<0.01
Total human albumin (100 mL)[a]	0.07±0.5	0.81±1.8	<0.01
Early OHSS rate (%)[β]	83.5	55.3	0.01
Late OHSS rate (%)[β]	16.5	44.7	0.01
Second admission (%)[β]	17.5	7.9	0.08

Values are mean ± standard deviation
[a]Non-parametric tests were used
[β]Chi-square tests were used, for all others, student's t-test was used
n: Number of patients, IVF: *In vitro* fertilization, hCG: Human chorionic gonadotropin, OPU: Oocyte pickup, Hb: Hemoglobin, Hct: Hematocrit, PLT: Platelet, HES: Hydroxyethyl starch, OHSS: Ovarian hyperstimulation syndrome

catheterization was 1-9 days in the catheterized group (mean: 2.9±1.7 days). After being discharged from hospital, 27 (14.3%) patients needed to be readmitted to hospital due to OHSS. In the catheterized group, the readmission ratio was 7.9%, whereas in the non-catheterized group, the readmission ratio was 17.5% (p=0.08) (Table 1).

The ratios of singleton, twin, and triplet pregnancies in the two groups were statistically similar, but the twin pregnancy ratio was higher in the catheterized group (Table 2). The miscarriage ratios, whether early miscarriage or chemical abortus, were statistically similar between the two groups (p>0.05) (Table 2).

The clinical pregnancy ratio of the catheterized group was higher than that of the non-catheterized group (p<0.01). In addition, the catheterized group had a statistically higher live birth ratio (p=0.04) (Table 2). The early OHSS ratio was higher in the non-catheterized group, and the late OHSS ratio was higher in the catheterized group (p=0.01).

The perinatal outcomes of the two groups, such as preterm labor, GDM, preeclampsia, PPROM, IUGR, and miscarriage ratios, were similar (Table 2).

The birth weights and gestational ages of the twin and singleton pregnancies were compared. The mean birth weight and gestational age at delivery of twins in the catheterized group were 2085 g and 34.6 weeks, respectively; in the non-catheterized group, these values were 2200 g and 35 weeks, respectively (p=0.65). The mean birth weight and gestational age at delivery of singleton babies in the catheterized group were 3251 g and 37.6 weeks, respectively; in the non-catheterized group, these values were 2728 g and 36.6 weeks, respectively (p=0.08) (Table 2).

Regression analyses of live birth rates of the two groups were performed, the catheter presence and other factors did not affect the live birth rates of the two groups (p>0.05) (Table 3).

Discussion

In our study, we compared the complications and obstetric outcomes of patients who had pigtail catheters applied for ascites drainage with those of patients who did not require catheterization. Pigtail catheter application, although conducted under the guidance of ultrasonography, is still an invasive method. During the procedure, the abdominal wall is punctured, which can cause peritoneal irritation or secondary infections related to the process itself, possibly resulting in fetal or maternal complications. We encountered no complications related to pigtail catheter application, such as infection, or bowel or other intra-abdominal organ trauma. We know that

Table 2. Obstetric outcomes of the two groups

	Non-catheter group	Catheter group	p
Ratio of pregnancy (n) (%)	71 (62.8)	56 (91.8)	<0.01
Ratio of abortus (n) (%)	15 (13.2)	16 (26.2)	0.09
Chemical pregnancy	2 (2.8)	3 (5.3)	0.17
Miscarriage	13 (18.3)	13 (23.2)	0.18
Ratio of live birth (n) (%)	56 (49.5)	40 (65.5)	0.04
Singleton pregnancy	40 (56.3)	25 (44.6)	0.20
Twin pregnancy	15 (21.1)	14 (25)	0.50
Triplet pregnancy	1 (1.4)	1 (1.7)	1.00
Ratio of preterm labor (n) (%)	13 (18.3)	10 (17.8)	0.80
Singleton pregnancy	8 (11.2)	4 (7.1)	0.70
Twin pregnancy	4 (5.6)	5 (8.9)	0.57
Triplet pregnancy	1 (1.4)	1 (1.7)	1.00
GDM (n) (%)	6 (8.4)	1 (1.7)	0.23
Preeclampsia (n) (%)	3 (4.2)	2 (3.5)	1.00
Twin birth weight (g ± SD)[a]	2200±347	2085±544	0.65
Twin gestational age at delivery (wk ± SD)[t]	35±1	34.6±2	0.64
Singleton birth weight (g ± SD)[t]	2728±735	3251±281	0.08
Singleton gestational age at delivery (wk ± SD)[t]	36.6±2.7	37.6±0.6	0.32

[a]Non-parametric tests were used
[t]Student's t-test, all other tests were chi-square tests
n: Number of patients, GDM: Gestational diabetes mellitus, g: Grams, SD: Standard deviation, wk: Weeks

Table 3. Logistic regression of factors effecting live birth rates

	p	Odds ratio	95% Confidence interval	
			Lower	Upper
Age	0.80	1.022	0.859	1.215
Total dose of gonadotropins used in IVF	0.38	1.000	1.000	1.001
Estradiol levels on day of hCG	0.64	1.000	1.000	1.000
Oocyte count obtained after OPU process	0.43	0.956	0.853	1.071
Oocyte count obtained after OPU process	0.52	1.045	0.912	1.198
Infertility duration	0.17	0.896	0.764	1.050
Hb count on first day of hospitalization	0.39	0.592	0.177	1.978
Hct ratio on first day of hospitalization	0.44	1.180	0.773	1.802
PLT count on first day of hospitalization	0.87	0.999	0.987	1.011
Hb count on last day of hospitalization	0.05	3.372	1.002	11.348
Hct ratio on last day of hospitalization	0.05	0.648	0.414	1.014
PLT count on last day of hospitalization	0.74	1.002	0.989	1.015
Length of hospitalization	0.06	1.434	0.972	2.115
Total HES solution[a]	0.08	0.759	0.556	1.037
Total human albumin[a]	0.33	0.766	0.446	1.314
Catheter[a]	0.68	1.357	0.307	5.997

[a]Categorical value
IVF: *In vitro* fertilization, hCG: Human chorionic gonadotropin, OPU: Oocyte pickup, Hb: Hemoglobin, Hct: Hematocrit, PLT: Platelet, HES: Hydroxyethyl starch

the inflammatory process can cause the release of cytokines and chemokines that can precipitate preterm labor[5].

Obstetric outcomes such as preterm labor, early and late miscarriages, preeclampsia, GDM, PPROM, IUGR, and fetal anomaly were compared between the two groups, and we found no differences. The analysis of miscarriage rates, including the subgroups early, late, and chemical miscarriage, also revealed similarities between the two groups (Table 2). Overall, the miscarriage ratio of the patients who had ascites drainage with pigtail catheter was 26.2%. The miscarriage ratio which was 13.2% in the control group was lower than the catheter group, but the difference was not statistically significant. The higher ratios of miscarriage could be explained by higher rates of OHSS ratios in the catheterized group than in the control group. Most of the patients who were hyperstimulated had polycystic ovaries and the miscarriage rate after IVF treatment is higher in patients with polycystic ovary syndrome (PCOS)[6,7]. In our groups, the numbers of patients with PCOS were similar; the higher ratios of miscarriage was not higher than the miscarriages rates of other studies such as Chen et al.[8] (26.6%), Serdyńska-Szuster et al.[9] (26.9%), and Raziel et al.[1] (38%).

Multiple pregnancy is a risk factor for preterm labor; however, although not statistically significant (p=0.06), the rate of twin pregnancy was higher in the pigtail group. The preterm labor ratios did not differ between the groups (p=0.28). In the literature, the preterm labor rate in patients with OHSS after IVF treatment varies from 1% to 28%[10-12]. In the study by Haas et al.[13], pregnancy outcomes in patients with severe OHSS following ascetic drainage were evaluated, and their preterm labor risk was found as 12.5%. In our study group, the preterm labor risk was 18.5%, and that of the control group was 19% (p>0.05). However, the Haas et al.[13] study included a small case sample (n=16), they had no control group, and most importantly, the pregnancies were all singletons. Even though twins and triplets were included in our study, the overall preterm labor rate was not much higher than that of the Haas et al.[13] study.

In our study, there were no differences between the groups in terms of birth weight or gestation age at delivery. The results regarding pregnancy outcomes after OHSS in IVF treatment revealed no differences between the groups and they were better than those in the review of Raziel et al.[14].

The rates of risk factors for developing OHSS, such as age, diagnosis of PCOS, total dose of gonadotropins during controlled ovarian hyperstimulation, estradiol level on the day of hCG, and oocyte numbers, were similar between the two groups. However, the catheterized group had a longer duration of hospitalization, they required greater amounts of HES and human albumin solution, and their platelet levels were higher on the day of discharge from hospital. These results were not entirely unexpected, because

the ascites in the catheterized group were more severe, thus requiring the pigtail catheter. Although the pigtail group had a more severe form of OHSS, the mean duration of hospitalization, i.e. the recovery time from OHSS was 7.7 days; however, in the study of Nouri et al.[15], the recovery time from OHSS was 9 days. Even if the pigtail group had a longer hospitalization time than the control group, it was still lower than the cases in the study of Nouri et al.[15]. Application of a pigtail catheter may not increase the length of hospitalization, but the severity of ascites might slow the recovery process.

Then clinical pregnancy ratio, live birth ratio per cycle, and late OHSS ratios were higher in the pigtail group than in the control group ($p<0.05$) (Table 2). When we analyzed the regression analysis of factors that effected the live birth rate, we found no significance between them (Table 3). The higher late OHSS ratios in the pigtail group can be reasonably explained by the higher pregnancy rate. In addition, it is known that the pregnancy rate is higher among patients undergoing IVF/ICSI who had OHSS[1]. Although not statistically significant, the catheterized group had a lower rate of hospital readmission than the non-catheterized group, despite the fact that the catheters were used in patients who were more clinically severe. This finding suggests that in managing OHSS, use of the pigtail catheter has an advantage in that it can help decrease hospital readmission rates.

Study Limitations

The major limitation of our study is its retrospective nature. Pigtail application is an invasive procedure, as such we did not have the chance to randomize the patients who had severe OHSS. Abdominal paracentesis may be an alternative to the pigtail catheter, but abdominal paracentesis needs to be applied blindly everyday through the abdominal wall. Pigtail application for drainage of ascites is made only once, thus the single application may be an advantage over repetitive abdominal paracentesis, which has not been practiced in our clinic for many years.

Conclusion

The application of pigtail catheters did not decrease the pregnancy ratio or live birth ratio in patients with OHSS. In addition, the hospital readmission rate was lower. The obstetric outcomes were similar between both groups. Thus, percutaneous pigtail catheters can be used in the treatment of ascites in patients with OHSS without raising concerns regarding pregnancy outcomes.

Ethics

Ethics Committee Approval: The study was approved by the Başkent University Ethics Committee.

Informed Consent: Consent form was not need because of retrospective nature.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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

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Subclinical hypothyroidism: Is it important in intracytoplasmic sperm injection cycles?

Subklinik hipotiroidi: İntrasitoplazmik sperm enjeksiyonu siklusları için önemli mi?

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Abstract

Objective: To compare intracytoplasmic sperm injection (ICSI) outcomes of women with subclinical hypothyroidism with those of euthyroid women.
Materials and Methods: A retrospective case-control study was conducted. Out of 2529 ICSI cycles evaluated, 41 women with hypothyroidism, 28 women with hyperthyroidism, and 128 women with subclinical hyperthyroidism were excluded, and 2336 cycles were analyzed. Women were identified as having subclinical hypothyroidism (case group, n=105) in the presence of a thyroid-stimulating hormone level >4.5 mU/L and normal free T4 and compared with euthyroid controls (n=2231).
Results: The mean age, body mass index, day 3 follicle-stimulating hormone level, and antral follicle count of the study patients were similar to the control group (p>0.5). The cycle cancellation rate of the study group was similar to the control group (13.3% vs. 7.6%, p=0.1). The clinical pregnancy rate was 21.2% in the study group, which was significantly lower than the 35.8% in the control group (p=0.04). The take-home baby rate was also significantly lower in the study group compared with the control groups (13.5% vs. 31.4% respectively, p=0.01).
Conclusion: Both the clinical pregnancy rate and the take-home baby rate is lower in women with subclinical hypothyroidism at the time of ICSI cycle.
Keywords: Female, infertility, intracytoplasmic sperm injection, subclinical hypothyroidism, pregnancy rate

Öz

Amaç: İntrasitoplazmik sperm enjeksiyonu (ICSI) uygulaması sonuçlarının, uygulanan subklinik hipotiroidi bulunan kadınlar ile ötiroid olan olgularda karşılaştırılmasıdır.
Gereç ve Yöntemler: Retrospektif olgu-kontrol çalışması olarak araştırıldı. Uygulanan 2529 ICSI siklusu içinden hipotiroidisi olan 41 kadın, hipertiroidisi olan 28 kadın ve subklinik hipertiroidisi olan 128 kadın dışlandı, sonuçta 2336 siklus değerlendirildi. Tiroid stimüle edici hormon düzeyi >4,5 mU/L ve serbest T4 düzeyi normal olan kadınlar subklinik hipotiroidi (olgu grubu, n=105) olarak değerlendirildi ve ötiroid kadınlar (kontrol grubu, n=2231) ile karşılaştırıldı.
Bulgular: Subklinik hipotiroidi olgularının ortalama yaş, vücut-kitle indeksi ve 3. gün folikül uyarıcı hormon değerleri kontrol grubu ile benzerdi (p>0,5). Siklus iptal oranları da istatistiksel olarak benzerdi (%13,3'e %7,6, p=0,1). Subklinik hipotiroidi olgularında kontrol grubuna göre klinik gebelik oranı istatistiksel anlamlı olarak daha düşüktü (olgu grubu %21,2 ve kontrollerde %35,8 p=0,04). Sağlıklı bebek doğum oranı da subklinik hipotiroidi olgularında kontrol grubuna göre istatistiksel anlamlı olarak daha düşüktü (sırasıyla %13,5'e %31,4, p=0,01).
Sonuç: Hem klinik gebelik oranı hem de sağlıklı bebek doğum oranı ICSI uygulanan subklinik hipotiroidi kadınlarda daha düşük olarak saptanmıştır.
Anahtar Kelimeler: Kadın, infertilite, intrasitoplazmik sperm enjeksiyonu, subklinik hipotiroidi, gebelik oranı

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PRECIS: In this multi-centered retrospective case-control study, we aimed to determine intracytoplasmic sperm injection (ICSI) outcomes of infertile women with subclinical hypothyroidism versus euthyroid infertile women, including higher numbers of ICSI cycles.

Introduction

Various experimental cell culture studies involving human and some animal species have shown that thyroid hormones have, to some degree, a stimulatory effect on granulosa and/or thecal cells[1-3]. Therefore, it is anticipated that disturbances in this thyroid-ovarian interaction might exert a negative influence on pregnancy as previously shown[4,5]. In this manner, hypo- or hyper-thyroidism have their own ways of management; however, it is not clear for subclinical hypothyroidism, which is defined as a serum thyroid-stimulating hormone (TSH) above the defined upper limit of the reference range, with a serum free thyroxine (fT4) within the reference range[6]. It is rather a laboratory diagnosis because patients with subclinical hypothyroidism have no or fewer symptoms[6]. The routine serum assays used to rule out subclinical hypothyroidism are not recommended, except in some specific patient groups including pregnant women due to possible developmental problems of the fetus related to maternal high TSH levels[6]. However, a recent Cochrane database systemic review concluded that there was insufficient evidence to recommend the use of one intervention for clinical or subclinical hypothyroidism before or during pregnancy over another, for improving maternal, fetal, neonatal, and childhood outcomes[7].

In a recent study of limited numbers of pregnant women with subclinical hypothyroidism, T4 treatment was reported to result in similar clinical pregnancy rates per cycle but higher embryo implantation rates, live birth rates, and lower miscarriage rates compared with the no treatment group[8]. In contrast with this study, subclinical hypothyroidism and overt hypothyroidism was shown not to benefit from T4 treatment in terms of the clinical pregnancy rates per started cycle, implantation rates, and the live birth rates per started cycle compared with euthyroid controls[9]. Therefore, in this multi-centered retrospective case-control study, we aimed to determine intracytoplasmic sperm injection (ICSI) outcomes of infertile women with subclinical hypothyroidism versus euthyroid infertile women, including higher numbers of ICSI cycles.

Materials and Methods

Hospital records of infertile women who attended participant referral study centers of assisted reproduction therapy (ART) between 2014 and 2015 were analyzed retrospectively in this retrospective case-control study. Patients without laboratory studies of thyroid functions, those with missing data related to ART, and those with thyroid function abnormalities such as thyroid autoimmunity diseases other than subclinical hypothyroidism were not included in the study. Ovarian stimulations were performed using a combination of gonadotropin-releasing hormone agonist/antagonist and follicle-stimulating hormone (FSH)/human menopausal gonadotropin. When the leading follicle diameter was larger than 17 mm, human chorionic gonadotropin (hCG) was given. Oocytes were retrieved 35 h after hCG administration. Following oocyte preparation, insemination was performed using ICSI in all cases one hour later. After insemination, eggs were individually cultured until day 5 for blastocyst transfer.

Data related to ICSI cycles, patients' demographics, laboratory results, and pregnancy outcomes were compared between the two groups of infertile women; the study group included infertile women with subclinical hypothyroidism and the control group comprised women who were euthyroid. Women were identified as having subclinical hypothyroidism in the presence of a TSH level >4.5 mU/L and a serum fT4 within the reference range[6]. The patients with hyperprolactinemia had been treated with levothyroxine. Hyperprolactinemia is also common in subclinical hypothyroidism[10]; therefore, patients with both subclinical hypothyroidism and hyperprolactinemia were included in the study and were treated with levothyroxine and a dopamine agonist.

Statistical Analysis

Out of 2529 ICSI cycles with eligible data, 41 women with hypothyroidism, 28 women with hyperthyroidism, and 128 women with subclinical hyperthyroidism were excluded, and 2336 cycles were analyzed. The related statistical comparisons of groups were performed using the with ANOVA test and chi-square test, where appropriate. Correlation analyses were performed with Pearson's correlation. Statistical analyses were performed using SPSS statistics software (SPSS Statistics for Windows, version 17.0; SPSS Inc., Chicago, USA). The p value was set as <0.05 for significance.

Results

Of the included 2336 cycles, 105 women were identified as having subclinical hypothyroidism (study group) and 2231 women were included in the euthyroid control group. The mean age, body mass index, type and duration of infertility were similar between the groups; only causes of infertility differed between the groups (Table 1). Both the study group and control euthyroid group were statistically indifferent considering infertility analysis values including FSH, luteinizing hormone (LH), estrogen, total antral follicle count, and sperm analyses (Table 2). Of the cases with subclinical hypothyroidism, 46% (n=48) had associated hyperprolactinemia. The rate of patients with TSH values equal or higher than 10 was 11% (n=12). With the exceptions of significantly lower estradiol measurements and endometrial thickness on hCG day, the outcomes of controlled ovarian hyper-stimulation and ICSI of the study group were

Table 1. Demographics of the study population and causes of infertility

Variable	Subclinical hypothyroidism (n=105)	Euthyroid controls (n=2231)	p
Age	31.2±5.9	32.1±5.2	0.1
Body mass index	24.1±3	24.4±3.5	0.4
Duration of infertility	94.7±52.5	102.2±59.7	0.2
Secondary infertility	15 (14.3)	449 (20.1)	0.6
Cause of infertility			
Tubal factor	20 (19%)	565 (25.3%)	0.1
Endometriosis	3 (2.8%)	276 (12.3%)	0.003*
Anovulatory cycles	5 (4.7%)	569 (25.5%)	<0.001*
Male factor	40 (38%)	683 (30.6%)	0.1
Unexplained	37 (35.2%)	138 (6.1%)	<0.001*

*Statistically significant (p<0.05), chi-square test

similar to those of the control group (Table 3). The cycle cancellation rate of the study group was similar to the control group (13.3% vs. 7.6%, p=0.1). The clinical pregnancy rate was 21.2% in the case group, which was significantly lower than the 35.8% in the control group (p=0.04). The take-home baby rate was also significantly lower in the study group compared with the control group (13.5% vs. 31.4%, respectively; p=0.01) (Table 4). Miscarriage rates were also higher in the study group compared with the control group (36% vs. 24%); however, the result was not statistically significant.

When the outcomes of patients with subclinical hypothyroid with and without hyperprolactinemia were compared in terms ICSI cycle parameters and pregnancy outcomes, none was found as significantly different (Table 5). In the subgroup analysis of patients with subclinical hypothyroidism according to TSH level (<10 versus TSH ≥10) for the outcomes of ICSI and pregnancy outcomes, only significantly lower levels of estradiol on hCG day and a significantly higher rate of cycle cancellation were present in patients with TSH ≥10 (Table 6).

Table 2. Laboratory analyses of the women and men

Laboratory analysis	Subclinical hypothyroidism (n=105)	Euthyroid controls (n=2231)	p
FSH	7.6±2.5	7.1±2.7	0.1
LH	4.9±2.3	5.1±2.7	0.4
E2	44.2±22.9	45.7±30.1	0.6
Total antral follicle count	12.8±6.5	13.1±5.8	0.6
Total motile sperm count	51.1±23.7x10^6	47.8±20.4x10^6	0.1
Oligospermia (<20x10^6/mL)	10 (9.5%)	246 (11%)	0.6
Oligoasthenoteratospermia	31 (29.5%)	495 (22.1%)	0.07
Azoospermia	9 (8.5%)	188 (8.4%)	0.9

*Statistically significant (p<0.05), independent samples t-test
FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol

Table 3. Outcome of controlled ovarian hyperstimulation and intracytoplasmic sperm injection

Outcome	Subclinical hypothyroidism (n=105)	Euthyroid controls (n=2231)	p
Total FSH used	3003±1357	3154±1338	0.2
Antagonist cycles	18 (17.3%)	269 (12.8%)	0.2
hCG day estradiol	1760±1057	2227±1241	<0.001*
hCG day endometrial thickness	9.4±2.1	10.5±2.1	<0.001*
Failed to induce follicular growth	13 (12.4%)	113 (5.1%)	0.001
Number of oocytes retrieved	10.6±6	11.2±6.6	0.4
Number of MII oocytes	8.2±5.1	9.2±5.6	0.08
Cleavage rate	95.2±16.7	92.6±20.5	0.2
Fertilization rate	63.7±25.8	67.2±25.7	0.2
Grade 1 and 2 embryo count	2.4±0.9	2.5±1	0.3

*Statistically significant (p<0.05), independent samples t-test
FSH: Follicle-stimulating hormone, hCG: Human chorionic gonadotropin, MII: Metaphase II

Table 4. Cycle cancellation and pregnancy outcome

Variable	Subclinical hypothyroidism (n=105)	Euthyroid controls (n=2231)	p
Cycle cancellation rate	17 (16.1%)	257 (11.5%)	0.1
No dominant follicle	13 (12.4%)	113 (5.1%)	0.001*
Risk of ovarian hyperstimulation syndrome	0	16 (0.7%)	0.4
Fertilization failure	2 (1.9%)	83 (3.7%)	0.3
Others**	2 (1.9%)	45 (2%)	0.9
Pregnancy per embryo transfer cycle	17 (19.3%)	657 (33.3%)	0.006*
Clinical pregnancy rate	14 (15.9%)	574 (29.1%)	0.007*
Take-home baby rate	9 (10.2%)	436 (22.1%)	0.008*

*Statistically significant (p<0.05), chi-square test
**Others include failed to retrieve metaphase II oocyte, failed to retrieve sperm and failed cleavage

Table 5. Outcome of patients with subclinical hypothyroidism with and without hyperprolactinemia (>30)

Outcome	Subclinical hypothyroidism (n=57)	Subclinical hypothyroidism with hyperprolactinemia (n=48)	p
Total FSH used	3039±1462	2961±1233	0.2
hCG day estradiol	1821±878	1691±1238	0.5
Number of oocytes retrieved	10.6±6	11.2±6.6	0.4
Number of MII oocytes	8.2±5.1	9.2±5.6	0.08
Cleavage rate	95.2±16.7	92.6±20.5	0.2
Fertilization rate	63.7±25.8	67.2±25.7	0.2
Grade 1 and 2 embryo count	2.4±0.9	2.5±1	0.3
Number of oocytes retrieved	10.6±6	11.2±6.6	0.4
Cycle cancellation rate	8 (14%)	9 (18%)	0.5
Pregnancy per embryo transfer cycle	11 (22.4%)	6 (15.4%)	0.4
Clinical pregnancy rate	10 (20.4%)	4 (10.3%)	0.2
Take-home baby rate	6 (12.2%)	3 (7.7%)	0.4

FSH: Follicle-stimulating hormone, hCG: Human chorionic gonadotropin, MII: Metaphase II

Table 6. Intracytoplasmic sperm injection outcomes according to thyroid-stimulating hormone values in patients with subclinical hypothyroidism

Outcome	Subclinical hypothyroidism TSH <10 (n=93)	Subclinical hypothyroidism TSH ≥10 (n=12)	p
Total FSH used	2858±1124	4127±2299	0.1
hCG day estradiol	1878±1048	866±633	0.01*
Cycle cancellation rate	11 (11.8%)	6 (50%)	0.001**
Pregnancy per embryo transfer cycle	14/85	3/6	
Clinical pregnancy rate	12/85	2/6	
Take-home baby rate	7/85	2/6	

*Statistically significant (p<0.05), Mann-Whitney test
**Statistically significant (p<0.05), chi-square test
FSH: Follicle-stimulating hormone, hCG: Human chorionic gonadotropin, TSH: thyroid-stimulating hormone

Discussion

Experimental studies have demonstrated an interaction of thyroid hormones with ovarian function, and clinical studies have also reported negative effects of thyroid hormonal excess or defects with overt clinical signs and symptoms[1-5]. However, diagnosis of subclinical hypothyroidism depends on laboratory analysis rather than clinical symptoms; therefore, its possible effects on fertility status and pregnancy rates with ART need to be clarified. To achieve this aim, previous studies reported contrary results including improved pregnancy rates with T4 treatment or no change in pregnancy rates with T4 treatment, even in infertile patients with overt hypothyroidism[8,9,11-14]. In this multicenter retrospective study, the TSH cut-off value was set as 4.5 mIU/L for the diagnosis of subclinical hypothyroidism, as suggested previously by the consensus of the American Endocrine Society, the American Thyroid Association, and the American Association of Clinical Endocrinologists[6].

However, in a relatively recent study, two different cut-off values (2.5 mIU/L vs. 4.5 mIU/L) values were compared in terms of rates of clinical pregnancy, delivery or miscarriage in a large, retrospective cohort study of patients undergoing their first *in vitro* fertilization (IVF) cycle[11]. No statistical differences were found between the groups and it was suggested that lowering the TSH cut-off value would increase the diagnosis rate of subclinical hypothyroidism five-fold[11]. In our study, however, when we compared groups with TSH values of 4.5 to 9.9 or ≥10 mIU/L, we found a significant higher rate of cycle cancellations in patients with TSH ≥10 mIU/L. Nevertheless, the clinical pregnancy rates and take-home baby rate did not differ, in a similar manner with the previous study, which compared TSH values of 2.5 vs. 4.5 mIU/L[11]. In this present study, we used an ICSI population to determine the possible effects of subclinical hypothyroidism. Previously, it was shown that overt hypothyroidism was related with a decreased chance of achieving pregnancy following IVF, even with appropriate treatment[5]. In another study that determined outcomes of controlled ovarian hyper-stimulation in women with thyroid autoimmune disease, oocyte pickup and embryo transfer, the performance of recombinant-FSH was significantly poorer in patients with thyroid autoimmune disease[12]. When TSH values with a cut-off value of 2.5 IU/L were compared, significantly higher serum E2 concentrations was determined in those with <2.5 IU/L[12]. In our study, we found a similar significant difference when comparing TSH values of 4.5-9.9 vs. 10 or more IU/L. In our study, the rate of subclinical hypothyroidism was 5%; however, in a previous study, it was reported as high as 13.9%, which was a significantly higher rate compared with fertile patients (3.9%)[13]. In the present study, the euthyroid group had an incidental higher frequency of endometriosis, anovulation, and unexplained infertility, all of which are expected to decrease success rates in ICSI cycles by themselves. However, despite this, we found a significantly lower clinical pregnancy and take-home baby rate in the study group compared with the control group.

The findings of the current study and some other previous observations as mentioned above need to be verified with further epidemiologic and experimental studies. In a study that compared laboratory results of blood samples drawn every 10 minutes during a 24-h period for pulse analysis of LH, TSH, and prolactin, no difference was found between euthyroid hypothyroid patients or those with subclinical hypothyroidism[14]. It was concluded that corpus luteum insufficiency in female infertility could not be explained by subclinical hypothyroidism and thus should not be treated with L-thyroxin for fertility reasons[14]. However, another study concerning hypothyroidism in IVF cycles concluded that high circulating estradiol during superovulation for IVF increased the binding of thyroxin to thyroxin-binding globulin, resulting in relative hypothyroidism during a super-ovulation cycle in women taking thyroxin replacement therapy[15].

Study Limitations

Major limitation of this study is that it depends on retrospective data analyses, which necessitates to be confirmed by further well designed prospective clinical study regarding effects of subclinical hypothyroidism on ICSI cycles.

Conclusion

In conclusion, both the clinical pregnancy and take-home baby rate are lower in women with subclinical hypothyroidism at the time of ICSI cycle, regardless of T4 treatment.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ç., R.N.E., D.C.Ö., B.K., S.Ç., K.S., Concept: E.Ç., R.N.E., D.C.Ö., B.K., S.Ç., K.S., Design: E.Ç., R.N.E., D.C.Ö., B.K., S.Ç., K.S., Data Collection or Processing: E.Ç., R.N.E., D.C.Ö., B.K., S.Ç., K.S., Analysis or Interpretation: E.Ç., R.N.E., Literature Search: E.Ç., R.N.E., Writing: E.Ç., R.N.E.

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Fetomaternal outcomes in pregnant women with hepatitis E infection; still an important fetomaternal killer with an unresolved mystery of increased virulence in pregnancy

Hepatit E enfeksiyonu olan gebe kadınlarda fetomaternal prognozlar; halen önemli bir fetomaternal öldürücü hastalık ve çözümlenmemiş bir gizem olarak gebelikte virülans artışı

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Abstract

Objective: Hepatitis is a prevalent infection in developing countries. While hepatitis B and C are deepening their roots in the developed world, hepatitis A and E are common in the developing world. The uniqueness of hepatitis is in its transformation from a relatively self-limiting disease in the non-pregnant state, to a highly virulent disease during pregnancy.

Materials and Methods: This retrospective observational study was conducted in the Department of Obstetrics and Gynecology, King George's Medical University, Lucknow, for a period of six months from June 2016 to November 2016 [probably during an endemic peak of hepatitis E virus (HEV)] to observe the clinical outcomes in HEV-infected pregnant women.

Results: A total of 32 anti-HEV immunoglobulin M-positive pregnant women were included, and fetomaternal outcomes were analyzed. Hepatitis E positivity was significantly associated with maternal mortality, intrauterine demise with prematurity, and premature rupture of membranes was the most common fetal complication noted.

Conclusion: The difference in extent of virulence of infection and variations in maternal morbidity, mortality, and rates of intrauterine demise, signify the presence of some factors that play a role and need to be further studied and evaluated.

Keywords: Encephalopathy, hepatitis B, hepatitis E, jaundice, pregnancy

Öz

Amaç: Hepatit, gelişmekte olan ülkelerde sık görülen bir enfeksiyondur. Hepatit B ve C, köklerini gelişmiş dünyada derinleştirirken, hepatit A ve E gelişmekte olan dünyada sık görülmektedir. Hepatitin kendine özgü özelliği, gebelik olmayan durumda nispeten kendi kendini sınırlayan bir hastalıktan, gebelik esnasında bir şekilde yüksek düzeyde virülan bir hastalığa dönüşmesidir.

Gereç ve Yöntemler: Bu retrospektif gözlemsel çalışma, Haziran 2016 ile Kasım 2016 tarihleri arasında altı aylık bir sürede [muhtemelen hepatit E virüsünün (HEV) endemik olarak pik yaptığı dönemde] King George Tıp Üniversitesi (Lucknow), Kadın ve Doğum Hastalıkları Anabilim Dalı'nda, HEV ile enfekte olmuş gebe kadınlarda klinik prognozları gözlemlemek üzere yürütülmüştür.

Bulgular: Toplam 32 anti-HEV immünoglobulin M-pozitif gebe kadın çalışmaya dahil edilmiştir ve fetomaternal prognozlar analiz edilmiştir. Hepatit E pozitifliği, maternal mortalite ile anlamlı derecede ilişkili bulunmuştur ve en sık belirlenen fetal komplikasyonlar prematürite ile birlikte intrauterin ölüm ve erken membran rüptürü olmuştur.

Sonuç: Enfeksiyon virülansının derecesinde ve maternal morbidite, mortalite ve intrauterin ölüm oranlarında farklılıklar, etkili olabilecek ve daha fazla incelenmesi ve değerlendirilmesi gereken bazı faktörlere işaret etmektedir.

Anahtar Kelimeler: Ansefalopati, hepatit B, hepatit E, sarılık, gebelik

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PRECIS: Hepatitis E in pregnancy is a virulent disease with high fetal and maternal morbidity and mortality. The burden of the disease can be reduced by providing better sanitary services and clean drinking water to pregnant women.

Introduction

Jaundice in pregnancy is a known high-risk factor that increases fetomaternal morbidity and mortality. While hepatitis B and C are deepening their roots in the developed world, hepatitis A and E are still more common in the developing world. The uniqueness of hepatitis E lies in its transformation from a relatively self-limiting disease in the non-pregnant state to a highly virulent disease during pregnancy. Hepatitis E virus (HEV) belongs to genus Hepevirus and family Hepeviridae. The RNA genome remains enclosed within a capsid composed of one or possibly two proteins, but many questions remain regarding its antigenicity.

Endemics usually occur during the rainy season. Once ingested, the virus first infects the liver followed by viremia and shedding in stool. Liver injury coincides with an elevation of transaminases and the appearance of anti-HEV immunoglobulin (Ig) M. The mechanisms behind its aggressive course during pregnancy are still not clearly understood. Clinical presentation varies from asymptomatic infection to anicteric, icteric, and fulminant hepatitis. Common presenting symptoms include yellowing of the eye and urine, fever, chills, anorexia, nausea, and abdominal pain. Aminotransferases are markedly elevated and may precede the onset of symptoms. Unlike hepatitis B virus (HBV) and hepatitis C virus (HCV), HEV infections are not known to cause cirrhosis or hepatocellular carcinoma.

Pregnant women are more susceptible to infection by HEV and progression to fulminant hepatic failure with high mortality rates and preterm deliveries. The disease is amenable to being prevented by a better sanitation check because the virus has a feco-oral route of transmission.

The present study was planned to evaluate the extent of maternal and fetal morbidity and mortality encountered due to hepatitis E in a tertiary care centre in North India.

Materials and Methods

This retrospective observational study was conducted in the Department of Obstetrics and Gynecology, King George's Medical University, Lucknow, India, over a period of six months from June 2016 to November 2016, the endemic season for hepatitis E. All women with jaundice or history of jaundice in the present pregnancy who delivered during that period were recruited in the study. Jaundice was diagnosed by physical examination but a confirmation was made using liver function tests. The prevalence of jaundice in pregnancy was calculated along with percentages of hepatitis A, hepatitis B, hepatitis C positivity and hepatic encephalopathy. Hepatitis B and C positivity was diagnosed using and enzyme-linked immunosorbent assay and hepatitis A and E were confirmed through IgM positivity. Maternal and fetal outcomes were also noted from the labor registers and delivery records. The study was reviewed and given clearance by the institutional ethics committee.

Results

Total number of women who delivered during this six-month period was 3692, 177 (4.7%) of whom had jaundice at the time of delivery or during the antenatal period. The women with jaundice were studied in detail and data were classified patients who were HBV positive, hepatitis A virus (HAV) positive, HCV positive, HEV positive, and those who did not have positive viral markers but had jaundice due to other causes such as severe preeclampsia, sepsis, typhoid, dengue or no definite cause. The women who delivered were further classified by their age, antenatal care and registration, period of gestation at delivery, mode of delivery, antepartum or intrapartum fetal demise, preterm premature rupture of membranes, hepatic encephalopathy, and derangement in liver function test, platelets, and coagulation profile. The mean period of gestation at the time of onset of jaundice was 31.7±7.3 weeks.

The age of the patients ranged between 20-38 years with a mean age of 25 years. Table 1 shows the different causes of jaundice encountered in our study. The mode of delivery and fetomaternal outcomes were recorded as shown in Tables 2 and 3. A detailed description of the hepatitis E-positive pregnant women is presented in Table 4. About 90% of preterm deliveries were spontaneous, and 10% were medically induced due to comorbid indications.

Table 1. Different types of jaundice noticed in pregnant women

Total cases of jaundice	n=177	
Hepatitis B	54	30.5%
Hepatitis C	15	8.4%
Hepatitis E	32	18%
Hepatitis A	8	0.4%
Jaundice due to causes other than viral hepatitis	68	38%

Table 2. Mode of delivery in patients with jaundice in pregnancy

	HBV	HCV	HAV	HEV	Others
Total	54	15	8	32	68
LSCS	14	3	1	4	16
Vaginal	36	11	7	28	51
Instrumental	4	1	0	0	1

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HAV: Hepatitis A virus, HEV: Hepatitis E virus, LSCS: Lower segment cesarean section

Discussion

Viral hepatitis is one of the most common causes of jaundice encountered during pregnancy. Amongst all types of viral hepatitis, hepatitis E causes the most damage and is most prevalent in Asia and Africa[1]. The prevalence of the disease in the developed world is less and the difference is remarkable. Lachish et al.[2] in a 10-year retrospective analysis found only fifteen pregnant women infected with HEV in Israel. Five (33%) patients in their series resulted in fulminant hepatitis, and two patients underwent urgent liver transplantation. They had no mortality among the mothers and fetuses, even in cases that resulted in fulminant liver failure. Other industrialized countries also rarely encounter autochthonous cases of hepatitis E in pregnancy[3]. We should realize that the scenario in developing countries like India, Pakistan[4] and Bangladesh[5] needs to be identified where both prevalence, morbidity, and mortality is high, and advanced modalities of treatment such as liver transplantation are not freely available. The high prevalence is also a constant threat for pregnant women travelling from industrialized countries[6].

Disease outbreaks have shown to suddenly increase the number of maternal deaths as reported by Gurley et al.[7] who reported that a sudden increase in jaundice deaths due to fulminant liver failure in pregnancy was retrospectively investigated and anti-HEV IgM positivity was seen in the neighbours of those who died. An accidental sewage contamination of municipal water led to maternal deaths, increased miscarriages, and perinatal mortality.

Contrary to its relatively benign course in the non-pregnant state, the infection takes a significantly virulent turn during pregnancy. Systematic reviews and meta-analyses have shown that patients in later gestations, particularly in the third trimester of pregnancy with HEV positivity or with fulminant hepatic failure, were significantly associated ($p<0.05$) with maternal mortality and intrauterine fetal death (27.0%)[8].

HEV is chiefly an enterically transmitted virus, but other modes of transmission have also been proposed including person-to-person transmission, blood, and transplacental transmission[9]. Transplacental transmission is probably the cause behind the high incidence of abortion, preterm labor, and intrauterine demise associated with the disease. Interestingly, HEV replication has also been reported to occur in extra-hepatic sites such as the placenta[10].

Various Indian studies have confirmed the deadly nature and grievous impact of hepatitis E infection on maternal and fetal life. In our study period, owing to an endemic, 32 patients delivered who were positive with a documented report of anti-HEV IgM positivity. Of these, the majority (78%) had not received adequate antenatal care hospital prior to registration at our hospital. The majority of women with jaundice, including those who were hepatitis E-positive, delivered vaginally. Amongst the pregnant women affected by hepatitis E, 12 (37.5%) presented with intrauterine demise in the antepartum period, and 2 (6.2%) had an intrapartum intrauterine demise. The rate of preterm delivery was 71.9%, and 28.1% delivered before 28 weeks. Seven (21.8%) women resulted in hepatic encephalopathy, and 31.2% had a deranged coagulogram. There were seven (21.8%) maternal deaths. All these numbers were significantly higher than in other types of viral hepatitis. Fetomaternal outcomes were significantly better with HBV, HCV, and HAV hepatitis compared with HEV, as shown in Table 3.

Table 3. Distribution of different types of jaundice with fetomaternal outcomes

	HBV	HCV	HAV	HEV	Others
Total	54	15	8	32	68
Term	41	10	6	9	15
Preterm	13	5	2	23	53
PPROM	2 (3.7%)	0	1 (12.5%)	9 (28.1%)	2 (2.9%)
Preterm <28 weeks	1 (1.8%)	0	1 (12.5%)	9 (28.1%)	14 (20.5%)
Fetal outcomes					
Live births	49	13	6	18	42
Still births	5	2	2	14	26
Antepartum	3 (5.5%)	1 (6.6%)	2 (25%)	12 (37.5%)	18 (26.4%)
Intrapartum	2 (3.7%)	1 (6.6%)	0	2 (6.2%)	8 (11.7%)
Maternal outcome					
Maternal death	0	1 (6.6%)	0	7 (21.8%)	11 (16.1%)
HE	0	0	0	7 (21.8%)	14 (20.5%)
Coagulopathy	0	0	0	10 (31.2%)	10 (14.7%)

HBV: Hepatitis B virus, HCV: Hepatitis C virus, HAV: Hepatitis A virus, HEV: Hepatitis E virus, HE: Hepatic encephalopathy, PPROM: Preterm premature rupture of membranes

Table 4. Details of hepatitis E infected cases

Sn	POG at jaundice	Bil (d)	SGPT	SGOT	Platelet	Period of gestation	Mode of delivery	Fetal outcome	Maternal outcome
1	33 weeks	15	1670	1500	50.000	Preterm	Vaginal	Stillborn	**Expired**
2	35 weeks	12	1000	1000	30.000	Preterm	Vaginal	Stillborn	Normal
3	28 weeks 2 days	11	1100	1200	55.000	Preterm	Vaginal	Stillborn	**Expired**
4	31 weeks	18	3000	1900	25.000	Preterm	Vaginal	Stillborn	**Expired**
5	29 weeks 6 days	6	500	567	1 lacs	Preterm	Vaginal	Stillborn	Normal
6	28 weeks 2 days	4.5	28	55	90.000	Preterm	Vaginal	Stillborn	Normal
7	24 weeks 5 days	6.8	550	300	1.7 lacs	Term	Vaginal	Normal	Normal
8	28 weeks 3 days	12	2400	1542	2 lacs	Preterm	Vaginal	Stillborn	**Expired**
9	32 weeks 1 day	14	1158	1657	60.000	Preterm	Vaginal	Stillborn	Normal
10	27 weeks 2 days	1.7	34	33	2 lacs	Preterm	Vaginal	Stillborn	Normal
11	24 weeks 6 days	10	1009	493	30.000	Preterm	Vaginal	Stillborn	**Expired**
12	36 weeks 3 days	8.7	251	86	2 lacs	Preterm	Vaginal	Normal	Normal
13	33 weeks 6 days	6.7	281	129	2 lacs	Preterm	Vaginal	Normal	Normal
14	35 weeks 1 day	4.8	70	88	1.8 lacs	Preterm	Vaginal	Normal	Normal
15	33 weeks	18	2500	2000	250.000	Preterm	Vaginal	Stillborn	**Expired**
16	39 weeks 6 days	3	300	260	2 lacs	Term	Vaginal	Normal	Normal
17	24 weeks	20	17	63	30.300	Preterm	Vaginal	Stillborn	**Expired**
18	28 weeks	6	150	177	1.4 lacs	Preterm	Vaginal	Normal	Normal
19	Term	4	40	45	1.5 lacs	Term	Vaginal	Normal	Normal
20	31 weeks	0.4	41	72	1.9 lacs	Preterm	LSCS	Normal	Normal
21	38 weeks 4 days	7	550	400	90.000	Term	Vaginal	Normal	Normal
22	39 weeks 2 days	9	282	384	98.000	Term	LSCS	Normal	Normal
23	26 weeks 2 days	7.9	653	598	2 lacs	Preterm	Vaginal	Stillborn	Normal
24	37 weeks	7.3	249	99	1 lacs	Term	Vaginal	Normal	Normal
25	38 weeks 2 days	3	90	99	1.7 lacs	Term	Vaginal	Normal	Normal
26	35 weeks	5	115	150	1.2 lacs	Term	Vaginal	Normal	Normal
27	34 weeks 4 days	7	800	779	1 lacs	Preterm	Vaginal	Stillborn	Normal
28	40 weeks 1 days	6	90	88	2 lacs	Term	Vaginal	Normal	Normal
29	36 weeks 5 days	5	96	67	1.1 lacs	Preterm	LSCS	Normal	Normal
30	35 weeks	7	100	99	1 lacs	Term	LSCS	Normal	Normal
31	33 weeks 5 days	8	200	198	1.2 lacs	Preterm	Vaginal	Normal	Normal
32	31 weeks	7.8	680	400	1.8 lacs	Preterm	Vaginal	Normal	Normal

POG: Period of gestation, Bil: Bilirubin, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, LSCS: Lower segment cesarean section

On applying the unpaired t-test, we found that adverse fetal outcomes were significantly associated with rising serum bilirubin levels of more than 11 mg/dL, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) of more than 1000 IU/L, and a platelet count of less than 85.000 cells/mm^3, and this difference was statistically significant.

Maternal outcomes were also significantly poorer in cases of serum bilirubin of more than 14 mg/dL, SGPT more than 1600, and SGOT more than 1200 and platelets less than 59.000 cells/mm^3, as shown in Tables 5 and 6.

Looking at the fetomaternal outcomes at the time of delivery and applying the chi-square test, we found that preterm vaginal delivery had a statistically significant risk of poor fetal outcome, whereas it is was nonsignificant as far as poor maternal outcomes were concerned, as shown in Tables 7 and 8.

Similar poor outcomes have been reported by several authors. Sahai et al.[11] reported that viral hepatitis in pregnancy led to high maternal mortality and also fetal wastage, especially if women presented with features of encephalopathy, fulminant hepatic failure, and coagulopathy.

Shinde et al.[12] reported in their 2-year prospective study that 46.1% of pregnant patients developed encephalopathy compared with 34% in the non-pregnant group. Among the pregnant women, 67.3% survived and 32% died. In the non-pregnant group, most patients survived and only 9% died. This difference was statistically significant (p<0.01). Adverse fetal outcomes were seen in 71.1% of pregnant women with acute

Table 5. Comparison of various parameters with fetal outcome

	Fetal outcome	n	Mean	Standard deviation	p
POG at jaundice	Normal	18	32.8889	9.18694	0.331
	Stillborn	14	30.3000	3.80607	
Bil (d)	Normal	18	5.8611	2.23347	0.001*
	Stillborn	14	11.2214	5.44669	
SGPT	Normal	18	229.72	189.474	<0.001*
	Stillborn	14	1133.50	948.646	
SGOT	Normal	18	174.50	121.416	<0.001*
	Stillborn	14	956.21	688.345	
Platelet (in lacs)	Normal	18	1.5100	0.41292	0.002*
	Stillborn	14	0.8536	0.67467	

Applied unpaired t-test for significance, *Significant
POG: Period of gestation, Bil: Bilirubin, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase

Table 6. Comparison of various parameters with maternal outcome

	Maternal outcome	n	Mean	Standard deviation	p
POG at jaundice	Normal	25	32.5640	7.95811	0.247
	Expired	7	28.8714	3.68271	
Bil (d)	Normal	25	6.3440	2.94067	<0.001*
	Expired	7	14.8571	3.93398	
SGPT	Normal	25	332.32	320.066	<0.001*
	Expired	7	1670.86	1039.625	
SGOT	Normal	25	313.20	375.392	<0.001*
	Expired	7	1242.57	720.751	
Platelet (in lacs)	Normal	25	1.3992	0.51057	0.001*
	Expired	7	0.5929	0.63208	

Applied unpaired t-test for significance, *Significant
POG: Period of gestation, Bil: Bilirubin, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase

hepatitis E, including pre-term delivery in 23%, stillbirth in 23%, abortion in 3.8%, and intrauterine fetal death in 21.1% of patients.

Sultana and Humayun[13] reported in their two years' experience of 25 patients who had acute hepatitis E while one amongst them also had coexistent acute hepatitis A. Twenty-four hepatitis E positive (96%) patients presented in third trimester of pregnancy while one (4%) pregnancy ended in the second trimester as a missed miscarriage. Twenty-one (84%) babies were born alive, 18 (86%) of which were preterm. Perinatal mortality was 26%, which was contributed to by intrauterine deaths and early neonatal deaths in 3 (14%) cases each. In total there were 5 (20%) maternal deaths, 4 (80%) in postpartum period and 1 (20%) in the antepartum period due to fulminant hepatic failure.

A recent six-month study conducted by Singla et al.[14] reported 27 HEV-positive (36%) women amongst a total of 82 women with jaundice. All five maternal deaths that they reported were not registered anywhere for antenatal care prior to presenting at their hospital and had raised bilirubin of more than 15 mg/dL with deranged coagulograms, encephalopathy, and intrauterine fetal deaths. On analyzing the morbidity data, it was also found that HEV-positive women had poorer outcomes as compared with their hepatitis B surface antigen-positive counterparts. In a 3-year prospective study, Prasad et al.[15] reported 55 symptomatic women who were anti-HEV IgM-positive with maternal mortality of 5% and one antenatal death. Similar to our study, they also found prematurity and preterm rupture of membranes as the most common fetal complication.

In our experience, we found that 56% of women had normal outcomes, whereas the disease turned virulent in the rest with maternal death in 21.8% and fetal demise in 77.7%. What the factors are that govern the virulence of the disease and why virulence is typically increased in pregnant states remain unanswered questions. Bi et al.[16] found that pregnancy serum accelerated HEV replication by suppressing oestrogen receptors and type I interferon in the early stage of infection. A comparison has also been reported between the clinical and subclinical presentation of the disease in pregnancy so as to understand why the disease can sometimes be self-limiting while other times it is life threatening. Several concepts have been studied to look for the cause of heightened disease severity in pregnancy. Ramdasi et al.[17] showed antibody-dependent disease severity and impaired immune response in pregnant women with a differential elevation of cytokines in clinical and subclinical hepatitis E disease. The study by Pal et al.[18] compared immune parameters among pregnant women with acute hepatitis E versus non-pregnant patients with hepatitis E and found reduced production of T-helper 1 (Th1) cytokines and an increase Th2 cytokines in the pregnant HEV group, which indicated a peculiar pathogenesis in pregnancy.

It is thought that a super infection of hepatitis E in patients with hepatitis B worsens liver disease. Huang et al.[19] performed a two-year study to find the incidence of HEV infection among

Table 7. Relating mode and time of delivery with fetal outcome

			Fetal outcome		Total
			Normal	Stillborn	
Mode	PTVD		6	14	20
		% Fetal outcome	33.3%	100.0%	62.5%
	FTVD		8	0	8
		% Fetal outcome	44.4%	0.0%	25.0%
	LSCS		4	0	S4
		% Fetal outcome	22.2%	0.0%	12.5%
Total		Count	18	14	32

Applied χ^2 test for significance, p=0.001 considered significant
PTVD: Preterm vaginal delivery, FTVD: Full term vaginal delivery, LSCS: Lower segment cesarean section

Table 8. Relating mode and time of delivery with fetal outcome

			Maternal outcome		Total
			Normal	Expired	
Mode	PTVD		13	7	20
		% Maternal outcome	52.0%	100.0%	62.5%
	FTVD		8	0	8
		% Maternal outcome	32.0%	0.0%	25.0%
	LSCS		4	0	4
		% Maternal outcome	16.0%	0.0%	12.5%
Total		Total	25	7	32

Applied χ^2 test for significance, p=0.105 considered not significant
PTVD: Preterm vaginal delivery, FTVD: Full term vaginal delivery, LSCS: Lower segment cesarean section

HBV-infected pregnant women and found that HEV infection was not a common occurrence with hepatitis B. None of their 391 HBV-infected patients were anti-HEV IgM-positive. We also had only one patient with both HBV and HEV positivity; we found the patient did not have a very different course than others.

Vertical transmission and intra-family transmission has also been studied and is variously reported in literature. It was reported as low by Gu et al.[20], whereas Krain et al.[21] have raised doubts about it due to inadequate research in the area of vertical transmission of HEV from mother to child.

The present study illuminates various points including the immense burden of disease in peak season, the extent of virulence in the form of fetomaternal life, and the uncertainty of disease course. The strength of the study is in the large number of test-positive HEV-positive pregnant women whose outcomes were evaluated.

Study Limitations

The weaknesses of the study were the non-evaluation of virus positivity in neonates, plus the non-availability of immediate liver transplant for women with fulminant liver failure who might have been saved. Also, only symptomatic HEV-infected women were studied and asymptomatic women were missed because universal testing was not possible even in the endemic season due to resource constraints. The reasons of varied disease nature is a topic of future research, also it is important to time delivery after a test is determined positive because of the high incidence of intrauterine demise and prematurity.

Conclusion

Hepatitis E is a deadly fetomaternal disease. The disease picture shows immense variation from patient to patient, which requires further research so as to better ascertain which factors play a role. It accounts for a significant number of deaths and increases the maternal mortality rate of the country.

The World Health Organization (WHO) also recognizes the burden of the disease. Due to the lack of sufficient information on safety, immunogenicity, and efficacy in pregnant women, the present position statement of the WHO does not recommend use of vaccines in pregnant women, or children aged <16 years[22].

Unlike other diseases such as HIV, it also has a feco-oral route of transmission and hence the disease burden can be lessened by ensuring better sanitation and provision of clean drinking water for pregnant women.

Ethics

Ethics Committee Approval: The study was approved by the King George's Medical University but the institution committee did not mandate an approval number because it was a retrospective analysis.

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

Peer-review: External and internal peer-reviewed.

Authorship Contributions

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

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Summary of 2185 prenatal invasive procedures in a single center: A retrospective analysis

İnvaziv prenatal tanı yöntemleri uygulanan tek merkezli 2185 olgunun retrospektif analizi

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Abstract

Objective: To determine the frequency, indications, and outcomes of diagnostic invasive prenatal procedures (DIPP) performed in a university hospital.
Materials and Methods: This retrospective, observational study included 2185 cases of DIPP (chorionic villus sampling, amniocentesis, and cordocentesis) performed at the department of obstetrics and gynecology of a university hospital between 2010 and 2016. We included all DIPP cases performed between 11 and 24 weeks of gestation. We compared the different types of DIPP performed in our hospital.
Results: Two thousand one hundred eighty-five procedures were performed (1853 amniocenteses, 326 chorionic villus sampling, and 6 cordocenteses). The main indication for performing invasive procedures was abnormal results of aneuploidy screening for trisomy 21, followed by maternal age, and fetal structural abnormality. The fetal karyotype was altered in 154 (26.1%) cases. Trisomy 21 was the most common aneuploidy followed by trisomy 18, monosomy X, and trisomy 13. Fetal karyotype could not be revealed in 42 (2%) cases due to maternal contamination in 18 cases, inadequate sampling in 4 cases, and failure of cell culture in 27 cases. There were 2 pregnancy losses due to the invasive procedure (only in amniocentesis).
Conclusion: The ideal approach to pregnancies with a detected chromosomal abnormality should be tailored according to the individual choice of the couples regarding whether they decide for or against a child with a known chromosomal abnormality.
Keywords: Amniocentesis, chorionic villus sampling, cordocenteses, prenatal genetic diagnostic testing

Öz

Amaç: Çalışmamızın amacı, bir üniversite hastanesinde gerçekleştirilen tanısal invaziv prenatal girişimlerin (DIPP) sıklığını, endikasyonlarını ve sonuçlarını belirlemektir.
Gereç ve Yöntemler: Bu retrospektif, gözlemsel çalışma, 2010-2016 yılları arasında bir üniversite hastanesinin kadın hastalıkları ve doğum bölümünde yapılan 2185 DIPP olgusunu (koryon villus örneklemesi, amniyosentez ve kordosentez) içermektedir. Eleventh-24. gebelik haftaları arasında işlem yapılan tüm DIPP olgularını dahil ettik. DIPP olgularını gruplandırarak 3 grup olarak birbirleriyle karşılaştırdık.
Bulgular: İki bin yüz seksen beş işlem yapıldı (1853 amniyosentez, 326 koryon villus örneklemesi ve 6 kordosentez). İnvaziv prosedürlerin uygulanması için başlıca endikasyonlar sırasıyla, trizomi 21 anöploidi taramasında saptanan anormal sonuçları, maternal yaş ve fetal yapısal anomalilerdi. Fetal karyotip değişikliği 154 olguda (%26,1) izlendi. En sık rastlanan anöploidi trizomi 21 idi, bunu sırasıyla, trizomi 18, monosomi X ve trizomi 13 takip etti. On sekiz olguda maternal kontaminasyon, 4 olguda yetersiz örnekleme ve 27 olguda kültür üretilememesi nedeniyle 42 olguda (%2) fetal karyotip belirlenemedi. İnvaziv prosedür nedeniyle 2 gebelik kaybı vardı (sadece amniyosentezde).
Sonuç: Kromozom anormalliği saptanan gebeliklerde en uygun yaklaşım, ailelelerin kromozom anomalisi saptanan çocuğu isteyip istememesine göre yönetimin bireyselleştirilmesidir.
Anahtar Kelimeler: Amniyosentez, koryon villus örnekleme, kordosentez, prenatal genetik diagnostik test

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PRECIS: The ideal approach to pregnancies with a chromosomal abnormality should be tailored according to the individual choice of the couples regarding whether they decide for or against a child with a known chromosomal abnormality.

Introduction

Prenatal genetic diagnostic testing is intended to determine if a specific genetic disorder or condition is present in the fetus with as much certainty as possible. In contrast, prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. Physicians use various methods to determine high-risk pregnancies that are candidates for prenatal detection of chromosomal abnormalities (CA) with invasive diagnostic procedures. Autosomal aneuploidy (especially trisomy 21, 18, and 13) screening is the most common and cost-effective prenatal screening test. Currently, first-trimester prenatal screening with a combined test improves the detection of Down syndrome (DS) cases with up to 90% accuracy and a 5% false positive rate[1,2]. In some circumstances, maternal serum screening and/or sonography can be false positive and this may result in performing unnecessary invasive procedures. Obstetricians use various methods to attain fetal cells for diagnosis, including chorionic villus sampling (CVS), amniocentesis (AC), and cordocentesis (CC). Detection of smaller CA other than structural and numeric CA can be performed due to recent improvements in medical genetics. Molecular DNA techniques allow detection of numerous single-gene disorders[3]. There is also a revolutionary development in prenatal screening that helps obstetricians screen an extensive scale of genetic disorders with a cell-free DNA technique.

Our aim was to investigate the indications, types of genetic techniques, fetal karyotype results, and pregnancy outcomes of women in a university hospital for performing invasive diagnostic procedures over a period of 7 years.

Materials and Methods

This is a retrospective cohort study of pregnant women who underwent genetic invasive procedures (AC, CVS or CC) between January 2010 and January 2017 at a university hospital, in the department of obstetrics and gynecology. The institutional ethics committee of our university hospital approved the study (approval number: 2017/12). A total of 2185 prenatal samples were included in the study. All patients signed an informed consent form following a genetic consultation and during which the risks, benefits, and limitations associated with screening and invasive tests were explained. We performed a sonographic examination of all patients to detect any morphologic abnormalities prior to invasive procedures. CVS, AC, and CC were performed between 11 and 14 weeks of gestation, from 15 weeks, and from 21 weeks, respectively. The same obstetricians performed all invasive procedures. Indications for prenatal diagnostic testing were: maternal age, abnormal results of aneuploidy screening (combined first-trimester or biochemical second-trimester screening for DS >1/300 or 1:150 for trisomy 13 or 18), abnormal structural findings in fetal ultrasound (including increased nuchal translucency ≥3.5 mm before the introduction of the first-trimester screening, major fetal abnormality, and soft ultrasound markers), parental translocation carrier status, parental carrier status of a known genetic disorder, previous child with aneuploidy or other genetic disorder, and maternal request. Demographic parameters regarding maternal characteristics such as age, gravidity, parity, and gestational age were recorded. Indications for prenatal invasive procedures, types of invasive procedures and genetic laboratory tests performed, and finally, any complications related to procedures were analyzed. The methods used for the purpose of prenatal diagnosis were conventional cytogenetic techniques (G-banding preparations), fluorescence *in-situ* hybridization (FISH), quantitative fluorescence polymerase chain reaction (QF-PCR), and molecular DNA techniques. FISH and QF-PCR were performed for rapid identification of trisomies (13, 18, and 21). We used G-banding for chromosomal karyotyping in patients at risk of aneuploidy, and DNA testing for specific mutations that cause disease in patients at risk of a genetic disorder.

The analysis of chromosomal aberrations was classified as numeric (autosomal trisomy -21, 18, 13, 17, 7-, monosomy, triploidy, and sex CA), structural (inversion, deletion, de novo marker, Robertsonian translocation, reciprocal translocation, chromosomal variant), and single gene disorders (fragile X syndrome, maple syrup disease, spinal muscular atrophy, congenital adrenal hyperplasia, thalassemia). Chromosomal mosaicism was also included in structural chromosomal aberrations.

We performed a standard procedure that consisted of an ultrasound-guided transabdominal approach using an 18-G needle for CVS and 20-G needle for AC. We discarded the first 1-2 mL of the AC specimen and dissected chorionic villi from maternal decidua carefully to avoid maternal cell contamination. The mean amount of amniotic fluid obtained using AC was 16-20 mL.

Results

There were 2185 invasive procedures, which consisted of 1853 AC, 326 CVS, and 6 CC over a period of seven years. Two thousand one hundred eighty procedures were performed in singleton pregnancies and 5 in twins. The performed invasive tests with regard to indications are shown in Table 1 and the summary of CA and genetic disorder rates is shown in Table 2. The most common indication for prenatal invasive procedures was abnormal results of aneuploidy screening for trisomy 21, followed by maternal age, and fetal structural abnormality.

Fetal karyotype could not be revealed in 42 (2%) cases due to maternal contamination in 18 cases, inadequate sampling in 4 cases, and failure of cell culture in 27 cases. A second sampling procedure was performed and a normal karyotype was revealed in seven cases with a failure of cell culture at the first procedure. The genetic laboratory techniques performed in our cases for testing of fetal samples were: conventional cytogenetic analysis (n=1974, 90.5%), QF-PCR (n=163, 7.5%), FISH (n=4, 0.2%), and molecular DNA testing (n=38, 1.7%). There were 154 (7%) cases with chromosomal structural or numeric abnormalities and 15 (0.7%) cases with a genetic disorder. An analysis of cases with CA revealed 145 results with disease-causing CA and 9 with chromosomal variants. The summary of CA diagnosed in our study is shown in Table 3. The most common numerical CA was trisomy 21 (73/2185; 3.3%), the most common structural CA was reciprocal translocation (13/2185; 0.6%) and chromosomal inversion (13/2185; 0.6%). There were two cases of trisomy 21 fetal karyotype coexistence with Robertsonian translocation in one case and Klinefelter sex CA in another case. The outcomes of pregnancies associated with CA and genetic disorders are listed in Table 4. Finally, there were 2 pregnancy losses due to the invasive procedures (only in AC).

Discussion

CA of the fetus can result in numerous complications, including abnormal phenotype (1/150 of live births)[3], miscarriage in the

Table 1. Invasive test methods with regard to the indications

Indications	Invasive methods			
	Chorionic villus sampling	Amniocentesis	Cordocentesis	Total
	n (%)	n (%)	n (%)	
Maternal age	24 (7.4)	222 (12.0)	0	246
Abnormal results of aneuploidy screening (TR 21)	159 (49.1)	1349 (72.8)	1 (16.7)	1509
Abnormal results of aneuploidy screening (TR 18)	8 (2.5)	79 (4.3)	0	87
Fetal structural anomaly	100 (30.9)	126 (6.8)	3 (50)	229
Parental translocation carrier	0	1 (0.1)	0	1
Parental carrier of genetic disorder	8 (2.5)	18 (1.0)	0	26
Previous fetus or child with aneuploidy	11 (3.4)	27 (1.5)	0	38
Previous child with genetic disorder	11 (3.4)	11 (0.6)	1 (16.7)	23
Maternal request	1 (0.3)	14 (0.8)	1 (16.7)	16
Previous child with structural anomaly	2 (0.6)	3 (0.2)	0	5
Parental aneuploidy	0	4 (0.2)	0	4

TR: Trisomy

Table 2. Summary of chromosomal abnormalities and genetic disorder rates

Indications	Diagnosis			
	Chromosomal abnormality		Genetic disorder	
	Count (n)	Column (%)	Count (n)	Column (%)
Maternal age	22	14.3	0	0.0
Abnormal results of aneuploidy screening (TR 21)	73	47.4	0	0.0
Abnormal results of aneuploidy screening (TR 18)	4	2.6	0	0.0
Fetal structural anomaly	52	33.8	0	0.0
Parental carrier of genetic disorder	0	0.0	8	5.3
Previous fetus or child with aneuploidy	2	1.3	0	0.0
Previous child with genetic disorder	0	0.0	7	46.7
Maternal request	1	0.6	0	0.0

TR: Trisomy

first trimester (50% of recognized miscarriages), and stillbirth in the second trimester (5% of stillbirths)[4]. Detection of CA in the prenatal period may help to decrease these complications. Currently, prenatal screening and/or diagnostic testing for aneuploidy is offered irrespective of age or risk[5]. In obstetric practice, an abnormal result of aneuploidy screening replaced maternal age (35 years or over) as the most common indication for invasive procedures. Besides, structural abnormalities comprise up to 12% of indications in most studies[6,7]. In our study, consistent with the literature, the most common indication for invasive procedures was an abnormal result of aneuploidy screening for trisomy 21, which was followed by maternal age and fetal structural abnormality, with rates of 69%, 11.25%, and 10.5%, respectively. These indications can vary, probably due to factors such as a difference between public health policies for screening CA, difficulties in accessing

Table 3. Summary of the chromosomal abnormalities diagnosed in our study

Numerical anomalies	CVS	Amniocentesis	Number
47,XN, +21	25	48	73
47,XN, +18	11	13	24
47,XN, +13	2	1	3
45,X	3	1	4
47,XN, +17	1	1	2
47,XN, +7	1		1
47,XXX		2	2
47,XXY (Klinefelter syndrome)*	2		2
69,XXX		1	1
Structural anomalies			
Reciprocal translocation	46,XN, t(2;11)(q35;q25) 46,XN, t(1;16)(p13.3;p13) 46,XN, t(1,10)(P34,P13) 46,XN, t(3;12)(p12;q24.3), pat	46,XN, t(9;20)(p13;q15.2) 46,XN, t(4;6)(q21;p21) 46,XN, t(11;22)(q13.2;q11.2) 46,XN, t(12;18)(p11.2;q11.2) 46,XN, t(1;3)(q23;21) 45,XN, dic(14;22)(p11.2;p11;2) 46,XN, t(1;16)(p13.3;p13) 45X, t(13-14)(q10,q10) 46,XN, t(5;21)(13;q22)	13
Robertsonian translocation*		46,XN, rob(13,14)(q10,q10) 45,X, rob(14;21)(q10;q10)	2
Deletion		46,XN, del(Y)(q12) 46,XN, del (X)(pterâ?p21.2)	2
Inversion	46,XN, inv(9)(p11,q13) x2	45,X, inv(9)(p12,q13) 46,XN, inv(9)(p12q13) 46,XN, inv(9)(p11,q13) x8 46,XN, inv(12)(p11.2;q13)	13
sSMC		47,XN, +mar	1
Variant	46,XN, 1qh+	46,XN, 15ps(+) 46,XN, 22ps(+) 46,XN, 22ps+, mat. 46,XN, 9qh+ 46,XN, 22pss x2 46,XN, 16qh+ x2	9
Mosaicism		46,XX/45,X x2	2

*Trisomy 21 fetal karyotype coexistence
sSMC: Small supernumerary marker chromosomes, CVS: Chorionic villus sampling

Table 4. The outcomes of pregnancies associated with chromosomal abnormalities and genetic disorders

	Diagnosis			
	Chromosomal abnormality		Genetic isorder	
Pregnancy outcome	Count	Column n (%)	Count	Column n (%)
Legal induced abortion associated with aneuploidy	73	66.4	0	0.0
Legal induced abortion associated with fetal structural anomaly	2	1.8	0	0.0
Declined abortion and continue pregnancy	35	31.8	1	50.0
Legal induced abortion associated with genetic disorder	0	0.0	1	50.0

obstetric care, fear of adverse results related to the procedures, and absence of treatment after certain diagnoses. The rates of other indications were also compatible with the literature[8,9]. Our study demonstrated a CA rate up to 7% (154/2185)[7,10,11]. Another study in a Turkish population focused on the same issue and revealed a CA rate of 4.4%[12]. The medical literature reports culture media failure lower than 1.0%[11,13-15] and we observed approximately the same rate (1.2%). The patients and their spouses accepted the second attempt in only 7 of 27 cases (6 CVS and one AC) and a new AC was performed, all of which revealed a normal karyotype.

In cases of CA incompatible with extra-uterine life, awareness of this fetal karyotype has allowed women and their spouses to make decisions about their pregnancies if they prefer a legal induced abortion. As a secondary benefit, the knowledge of fetal karyotype also helps to alleviate anxiety throughout the pregnancy related with abnormal screening results. We preferred AC over other invasive procedures due to their diagnostic reliability, ease of the procedure, late obtainment of results, and a relatively low fetal loss rate. A current estimate of invasive procedure-related loss in experienced hands for AC and CVS are 0.1% and 0.2%, respectively[16]. We performed CC in a limited number of circumstances due to concerns of a higher risk of miscarriage and late obtainment of results. In general, CVS is less preferred than AC, which leads to a slightly higher risk of miscarriage, and possible false-positive results due to confined placental mosaicism (usually requires confirmatory diagnosis with AC).

Cytogenetic analysis deals with viable cells obtained from CVS, AC or CC, whereas DNA molecular testing uses either viable or non-viable cells for analysis. There are many different laboratory techniques that can be used for testing fetal samples: karyotyping, FISH-interphase, chromosomal microarray analysis (CMA), and molecular DNA testing. Analysis of cultured fetal cells allows detection of CA larger than 5-10 Mb[3]. In addition, CMA enables better determination of minor CA and microdeletions by increasing the detection rate by about 2.9% as compared with conventional karyotyping[17]. CMA is used on very limited occasions in a restricted number of laboratories and is not accessible with cost-effective prices in Turkey. We do not use this method in our clinic. Neither CMA nor FISH examinations can detect non-disjunction and translocation aneuploidy. Balanced translocations are usually associated with normal phenotype and may have serious complications including recurrent miscarriage and an increased risk of having abnormal offspring[3,18]. In the present study, 16 translocations (14 reciprocal and 2 Robertsonian) were detected. The risk of a serious congenital anomaly in such translocations is expected as 3.7% for Robertsonian translocations, 6.1% for de novo reciprocal translocations, and 9.4% for inversions[18]. Triploidy is incompatible with life; very few fetuses have lived beyond 20 weeks of gestation in the literature. Early termination of pregnancy restricts complications and maternal mortality associated with triploidy[19]. There was only one triploidy in our study and we induced abortion after genetic counseling. A rapid, economic, and accurate diagnosis of common aneuploidies (trisomy 21, 18, 13) is available with QF-PCR. Unfortunately, some chromosome aberrations cannot be diagnosed using QF-PCR, as such, this technique cannot be solely used instead of conventional cytogenetic analysis[20,21]. In our study, we performed QF-PCR more commonly in comparison with FISH analysis for rapid identification of trisomy (13, 18 and 21) beyond 20 weeks of gestation.

Chromosomal mosaicism is defined as the existence of different cell types in a tissue with a rate of 0.25% for AC and 1% for CVS[22,23]. In our study, we diagnosed two cases of mosaicism following AC and abortion was induced in both after genetic counseling. This low rate of mosaicism in our study may have resulted from the high AC preference with regard to CVS. The skill and practice of the obstetrician plays an important role in performing invasive procedures[14,15,24]. In our study, we observed only 2 abortions (0.09%) associated with invasive procedures. Our obstetrics team is experienced with invasive procedures and the same physicians (authors of this study) always perform these procedures. This finding may have contributed to the very low procedure-related miscarriage rate as compared with the literature.

In summary, 4.7% of cases were detected as having chromosomal trisomy 13, 18 or 21 (102/2185), 2.4% (52/2185) of cases had other chromosomal rearrangements, and in 0.7% (15/2185) of cases, single gene disorders were present (Table 2).

In Turkey, pregnancies with genetic abnormalities can legally be terminated at up to 24 weeks of gestation. Patients and spouses were informed about the presence of a genetic abnormality

and 49% (76/154) of our patients requested dilatation and curettage. Genetic testing was repeated after induced abortion and the same genetic results were confirmed in all cases.

In contrast, 23% (36/154) of the patients declined abortion and resumed their pregnancy. The reasons for this rejection included religious beliefs, very desired pregnancies, or twin pregnancies (no option for selective abortion). We could not perform confirmatory genetic testing for the postnatal follow-up of cases in which abortion was declined. These patients were all referred from other hospitals and all were lost to follow-up. Patients lost to follow-up accounted for 27% (42/154) of patients with a genetic disorder.

The strengths of this report are the chromosomal abnormality-single gene disorder diversity and performance of invasive methods by the same obstetric team. As far as we know, this is one of the largest and most comprehensive studies reported from the Southeast Anatolian region, which may give crucial clues for a specific Turkish women population regarding prenatal genetic diagnosis.

Study Limitations

The retrospective nature and limited number of CC are limitations of our study.

Conclusion

In conclusion, women face many problematic issues while undergoing invasive procedures, including the possibility that the fetus will have a CA or single-gene disorder, the risk of procedure-related miscarriage, and the consequences of a child with CA. Improved laboratory facilities, more accessible high-technology ultrasound equipment, and developed cytogenetic technology (detection of microdeletions and microduplications) contribute to increased prenatal detection of chromosome abnormalities. The rate of CA of newborns can be decreased through prenatal detection of abnormal karyotypes, which may result in reduced social trauma and financial load on both the parents and society due to disabled children. The ideal approach to pregnancies with detected CA should be tailored according to the individual choice of the couples regarding whether they decide for or against a child with a known CA.

Ethics

Ethics Committee Approval: The study was approved by the Gaziantep University Local Ethics Committee (approval number: 2017/12).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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The effect of pelvic organ prolapse type on sexual function, muscle strength, and pelvic floor symptoms in women: A retrospective study

Pelvik organ prolapsus tipinin kadınlarda seksüel fonksiyon, kas kuvveti ve pelvik taban semptomları üzerine etkisinin incelenmesi: Retrospektif araştırma

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Abstract

Objective: This retrospective research was planned to investigate the effect of pelvic organ prolapse (POP) type on sexual function, muscle strength, and pelvic floor symptoms in symptomatic women.

Materials and Methods: Data on POP type and stages as assessed using the Pelvic Organ Prolapse-Quantification system of 721 women who presented to the women's health unit between 2009 and 2016 were collected retrospectively. POP types were recorded as asymptomatic, anterior, apical, and posterior compartment prolapses. Sexual function was assessed using the Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire short-form (PISQ-12), pelvic floor muscle strength was assessed through vaginal pressure measurement, and pelvic floor symptoms and quality of life were assessed using the Pelvic Floor Distress Inventory-20 (PFDI-20).

Results: Among 168 women who met the inclusion criteria, 96 had anterior compartment prolapses, 20 had apical compartment prolapses, 16 had posterior compartment prolapses, and 36 women were asymptomatic. There was no difference between the groups in their PISQ-12 total and subscales scores, PFDI-20 total and two subscale (colorectal/anal, urinary) scores, and muscle strength ($p>0.05$). In the Pelvic Organ Prolapse Distress Inventory-6, another subscale of PFDI-20, it was determined that there was a difference between asymptomatic women and those with anterior compartment prolapses ($p=0.044$) and apical compartment prolapses ($p=0.011$).

Conclusion: This research found that POP type did not affect sexual function, muscle strength, and colorectal and urinary symptoms in our cohort. There were more prolapse symptoms and complaints in women with anterior and apical compartment prolapses.

Keywords: Pelvic organ prolapses, sexual function, muscle strength, pelvic floor symptoms

Öz

Amaç: Bu retrospektif araştırma, pelvik organ prolapsus (POP) tipinin kadın seksüel fonksiyon, kas kuvveti ve pelvik taban semptomları üzerine etkisini incelemek amacıyla planlandı.

Gereç ve Yöntemler: 2009-2016 tarihleri arasında kadın sağlığı ünitesine yönlendirilen 721 kadının verileri retrospektif olarak değerlendirildi. Kadınların prolapsus tip ve evreleri Pelvik Organ Prolapsus-Sınıflaması testi ile değerlendirildikten sonra; asemptomatik, anterior, apikal ve posterior kompartman prolapsusu olarak kaydedildi. Seksüel fonksiyonları Pelvik Organ Prolapsus/Üriner İnkontinans Seksüel Değerlendirme kısa formu (PISQ-12) ile, pelvik taban kas kuvveti vajinal basınç ölçümü ile ve pelvik taban semptom, şikayet ve yaşam kalitesi Pelvik Taban Distres Envanteri-20 (PFDI-20) ile değerlendirildi.

Bulgular: Dahil edilme kriterlerini karşılayan 168 kadının 96'sında anterior, 20'sinde apikal, 16'sında posterior kompartman prolapsusu ve 36 kadının da asemptomatik olduğu saptandı. Gruplar arasında; PISQ-12 toplam ve alt skala puanlarında, PFDI-20 toplam ve iki alt skala (kolorektal/anal, üriner) puanlarında ve kas kuvvetinde fark bulunmadı (p 0,05). PFDI-20'nin diğer alt skalası olan Pelvik Organ Prolapsus Distres Envanteri-6'da ise asemptomatik kadınlar ile anterior kompartman prolapsusu (p=0,044) ve apikal kompartman prolapsusu (p=0,011) olanlar arasında fark olduğu saptandı.

Sonuç: Bu araştırma ile POP tipinin seksüel fonksiyonu, kas kuvvetini, kolorektal ve üriner semptomları etkilemediği bulundu. Prolapsus semptom ve şikayetlerinin, anterior ve apikal kompartman prolapsusu olan kadınlarda daha fazla olduğu saptandı.

Anahtar Kelimeler: Pelvik organ prolapsusu, seksüel fonksiyon, kas kuvveti, pelvik taban semptomu

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PRECIS: Pelvic organ prolapse type did not affect sexual function, muscle strength, and colorectal and urinary symptoms.

Introduction

American College of Obstetricians and Gynecologists defined pelvic organ prolapses (POP) as prolapsing of the organs in the pelvis from inside and outside of the vaginal canal downward[1]. POP is described as anterior, posterior, and apical compartment prolapses according to their location in the vaginal canal[2]. POP symptoms may vary and may not necessarily be specific to any one compartment. Anterior compartment symptoms are urinary frequency, urgency, incontinence, intermittent flow, urinary difficulty, sense of incomplete discharge in the urinary bladder, and insufficient flow; posterior compartment symptoms are defecation difficulty, sense of incomplete discharge in bowels, constipation, and digital palpation need for discharge[3,4]. The only accepted symptom of POP seen in the three compartments is vaginal protrusion[4]. It was reported that POP prevalence ranged between 6-97% and POP affected 50% of women who gave birth to various degrees and 20% of these were asymptomatic[4-7].

POP is a serious public health problem that affects sexual function, quality of life, and psychological state; however, it is generally ignored by women. It was reported in that POP affected sexual function and life quality of women negatively[8-11]. However, a limited number of studies have contributed data regarding how women are affected by POP in relation to the prolapse compartment[12,13]. The objective of this research was to investigate the effect of prolapse compartments on sexual function, muscle strength, pelvic floor symptoms, and quality of life in symptomatic women.

Materials and Methods

Participants

This study was conducted retrospectively on 721 women who presented to Abant İzzet Baysal University, School of Kemal Demir Physical Therapy and Rehabilitation, Women's Health Unit, between November 2009 and January 2016. The inclusion criteria for the research were determined as being clinically diagnosed with stage 1 and over POP, being aged more than 18 years, sexually active, and speaking Turkish. Women who had symptoms of urinary and fecal incontinence without POP, any mental problems that hindered comprehension, neurologic or psychiatric illness, pregnancy, pelvic surgery history, incomplete assessment form, and the same stage POP in more than one compartment were excluded. Approval from the institutional review board and informed consent form all the participants were obtained (approval number: 2014/17).

The women included in the research were divided into 4 groups; women with stage 2 and above POP symptoms: anterior, apical and posterior, and asymptomatic women with stage 1 prolapse. The physical features of the women [age, body height, body weight, body mass index (BMI)] and their sociodemographic information (educational status, profession, menstrual status, obstetric anamnesis, and medical history) were recorded. Pelvic Organ Prolapse-Quantification (POP-Q) assessment, pelvic floor muscle strength, Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire short-form (PISQ-12), and Pelvic Floor Distress Inventory-20 (PFDI-20) data were taken from the women's files.

Pelvic Organ Prolapse-Quantification

POP-Q assessments of the women were made by a physiotherapist specialized in urogynecologic physiotherapy. POP-Q is a quantitative standardized measurement method used for determining POP localization and level. After the women were placed in the lithotomy position, anterior (Aa and Ba), posterior (Ap and Bp), apical (C and D), total vaginal length (TVL), genital hiatus, and perineal body (PB) were measured using a rule and recorded in centimeters. In POP-Q assessment, with the exception for TVL, all values were recorded at maximal protrusion using the Valsalva maneuver. These measurement results were recorded in 3x3 table and POP staging was made. POP-Q stages range between 0 and 4, and a high stage indicates more serious prolapses[14]. The women were classified as having anterior, apical, and posterior compartment POP, and asymptomatic POP based on these results.

Pelvic floor muscle strength measurement

Pelvic floor muscle strength was assessed through vaginal pressure measurement (Myomed 932 Enraf/Nonius®). The women were positioned on their back with their hips and knees flexed. The contraction and resting periods of the device were adjusted to 10 seconds. After the placement of the vaginal probe into the vagina, the women were told to relax their pelvic floor muscles with the "relax" command, and to squeeze the placed vaginal probe and lift it inside without contracting their abdomen, hip and thigh muscles, and without holding their breath with the "contract" command. The measurement was repeated three times and pelvic floor muscle strength was recorded as hectopascal[15].

Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire-12

Sexual function of women who were sexually active during the past six months were assessed using the Turkish version of PISQ-12. PISQ-12 is a valid and reliable condition-specific questionnaire that assesses behavioral/emotive, physical, and partner-related aspects of sexual function. The questionnaire provides information about sexual desire and activity frequency and orgasmic characteristics. PISQ-12 assesses the effect of POP on sexual function, women's perception, and how partners view pelvic floor disorders. In addition, it questions sexual function of partners. The questions are scored between 0 (never) and

4 (always), and questions 5-12 are estimated inversely. The maximum score obtainable from each section is 16. The total score achievable in the questionnaire ranges between 0-48. A high score indicates better sexual function[16].

Pelvic Floor Distress Inventory-20

Pelvic floor distress symptoms, quality of life, and pelvic floor dysfunction severity were assessed using the reliable and condition-specific Turkish version of PFDI-20. PFDI-20 consists of 3 subscales as Pelvic Organ Prolapse Distress Inventory-6 (POPDI-6), Urinary Distress Inventory-6 (UDI-6), and Colorectal-Anal Distress Inventory-8 (CRADI-8), and 20 questions. The subscale scores of PFDI-20 are 0-100 and the total scores (sum of three subscale scores) range from 0-300; high scores indicate more severe pelvic floor distress[17].

Statistical Analysis

Sample size requirement was calculated using G*Power Manual 3.1.2.9[18]. According to the outputs, a total of 112 women were required to ensure 80% power with an alpha level 0.05, and effect size was taken at the 0.4 level. The descriptive values of the obtained measurements were estimated as mean, median value, standard deviation, number and frequency. Whether the numerical characteristics showed normal distribution in each group was examined using the Shapiro-Wilk test. The Kruskal-Wallis test and post-hoc Dunn test were used for group comparisons. The statistical significance level was accepted as $p \leq 0.05$ and the SPSS version 20-demo program was used in the estimations.

Results

Seven hundred twenty-one files were examined during this research. Three hundred sixty-one women among those were excluded for having urinary incontinence without POP, and 3 women were excluded for only having symptoms of fecal incontinence. When the remaining files were examined, it was determined that 357 women had POP. Among these, 5 women with mental problems, 64 women with the same stage POP in more than one compartment, 2 women who could not speak Turkish, 59 who had not had a sexual relationship within the past 6 months, 12 with neurologic disorders, 23 women with a history pelvic surgery, and 24 women whose assessment forms were incomplete were not included in the study. A total of 168 women, comprising 96 women with anterior compartment prolapse, 20 women with apical compartment prolapse, 16 women with posterior compartment prolapse, and 36 women with asymptomatic POP were included in the final analysis (Figure 1).

The physical and sociodemographic characteristics of the women included in the research are shown in Tables 1 and 2. There was no difference found in terms of age and height of asymptomatic women, women with anterior, posterior, and

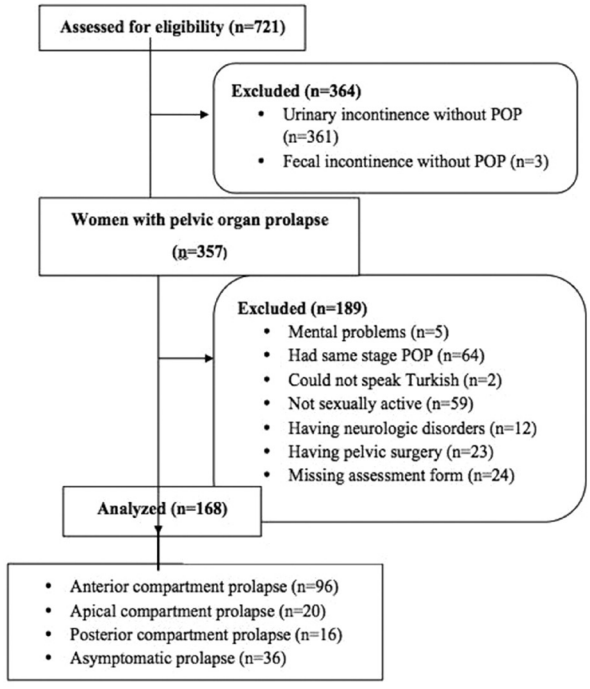

Figure 1. Enrollment diagram

POP: Pelvic organ prolapse

Table 1. Physical features of the women by prolapse type

	Anterior (n=96)	Apical (n=20)	Posterior (n=16)	Asymptomatic (n=36)	p[1]	Group comparison	p[2]
Age (years)	49.38±10.242	50.85±12.46	52.81±13.85	51.78±11.97	0.43		
Body height (m)	1.57±0.06	1.58±0.06	1.56±0.05	1.57±0.06	0.87		
Body weight (kg)	75.58±12.82	64.90±10.80	74.37±12.42	72.42±11.27	0.00*	Apical-anterior	0.00*
BMI (kg/m^2)	30.42±5.30	26.08±5.01	30.30±5.37	29.54±5.29	0.01*	Apical-anterior	0.01*

[1]Kruskal-Wallis test
[2]Post-hoc Dunn test
*$p \leq 0.05$
BMI: Body mass index

Table 2. Sociodemographic characteristics of the women by prolapse type

	Anterior (n=96)		Apical (n=20)		Posterior (n=16)		Asymptomatic (n=36)	
	n	%	n	%	n	%	n	%
Educational status								
Illiterate	9	9.4	4	20	2	12.5	4	11.1
Literate	7	7.3	1	5	0	0	1	2.8
Elementary school	65	67.7	10	50	9	56.3	23	63.9
High school	12	12.5	1	5	4	25	4	11.1
University	3	3.1	4	20	1	6.3	4	11.1
Profession								
Housewife	80	83.3	15	75	15	93.75	32	88.9
Working woman	16	16.7	5	25	1	6.25	4	11.1
Menstrual status								
Normal	29	30.2	6	30	6	37.5	11	30.6
Irregular	21	21.9	0	0	2	12.5	3	8.3
Menopause	46	47.9	14	70	8	50	22	61.1
Obstetric anamnesis	**Standard Error**	**Mean**	**Standard Error**	**Mean**	**Standard Error**	**Mean**	**Standard Error**	**Mean**
Gravida	0.178	3	0.260	2.5	0.579	2.5	0.324	2
Para	0.167	3	0.275	2.5	0.598	2	0.316	2
Number of live children	0.113	3	0.235	2	0.461	2	0.212	2

apical compartment prolapses (p>0.05). A statistical difference was determined in body weights (p=0.003) and BMI (p=0.011) of women with apical and anterior compartment prolapses.

There was no difference found between the groups in terms of PISQ-12 total scores and behavioral/emotive, physical, partner-related subscales, PFDI-20 total scores, and CRADI-8, UDI-6 subscales, and muscle strength (p>0.05, Table 3). POPDI-6 scores, a subscale of PFDI-20, exhibited statistically significant differences between asymptomatic women and women with anterior compartment prolapses (p=0.044) and apical compartment prolapses (p=0.011).

Discussion

This retrospective study, conducted to scrutinize the effect of POP type on sexual function of women, showed that different prolapse types did not affect sexual function. Sexual function has a critical role in women's health, interest, and awareness in this field has been increasing gradually[19]. Sexuality depends on several factors including body image, socio-emotional perspective, sexual perception, quality of relationship with partner, and partner's desire and competency[20]. It is known that POP mostly affects sexual function in the issues of sexual desire, orgasm ability, and arousal[10,21,22]. Psychological factors such as change in body image that could occur in women with POP, physiologic factors such as anatomic anomalies and diminished sensitivity in the genital region can lead to stimulation and orgasm disorders in women[12,23].

Studies examining sexual function based on prolapse types are scarce. Mouritsen and Larsen[13] showed that bladder, bowel, and sexual symptoms could be frequently seen in women with prolapses; however, they pointed out a very weak correlation between these symptoms and prolapse in a specific compartment. Lowenstein et al.[12] reported that sexual function was not related with POP stage and vaginal compartment types but more related with body image perception and distress caused by POP level in women, rather than topographic changes based on POP. Lowenstein et al.[12] concluded that women with poor body image had poor sexual functions. We found similar results in our research using the PISQ-12 to assess sexual function and by using the POP-Q system to determine prolapse type and stage. However, body image perceptions of these women were not able to be assessed due to the lack of a body image

Table 3. Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire-12, Pelvic Floor Distress Inventory-20 and muscle strength assessment by prolapse type

	Anterior (n=96)	Apical (n=20)	Posterior (n=16)	Asymptomatic (n=36)	p[1]	Group comparison	p[2]
PISQ-12	29.61±7.24	29.60±6.38	32.68±4.94	30.00±7.05	0.406		
Behavioral/Emotive	5.65±3.68	4.40±4.28	5.56±3.01	5.05±3.85	0.329		
Physical	12.37±3.92	12.75±3.94	14.68±1.77	13.50±3.33	0.097		
Partner related	11.58±2.71	12.45±2.32	12.43±2.06	11.44±3.12	0.542		
PFDI-20	87.25±60.39	79.78±54.08	83.52±47.19	67.73±53.29	0.313		
POPDI-6	27.64±24.09	33.53±28.56	24.21±21.09	16.08±20.04	0.029*	Asymptomatic-anterior Asymptomatic-apical	0.044* 0.011*
CRADI-8	18.81±19.12	15.62±17.73	30.66±20.67	19.35±20.60	0.119		
UDI-6	40.79±29.25	30.62±24.11	28.64±18.99	32.28±28.56	0.204		
Muscle strength (hPa)	21.83±13.55	15.00±9.07	23.43±15.70	23.33±14.42	0.158		

[1]Kruskal-Wallis test
[2]Post-hoc Dunn test
*p≤0.05
PISQ-12: Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire-12, PFDI-20: Pelvic Floor Distress Inventory-20, POPDI-6: Pelvic Organ Prolapse Distress Inventory-6, CRADI-8: Colorectal-Anal Distress Inventory-8, UDI-6: Urinary Distress Inventory-6, hPa: Hectopascal

questionnaire specific to prolapse in the literature during the period when data were collected.

The behavioral/emotive subscale of sexual function did not alter according to POP types in this study. Nevertheless, the scores received in the behavioral/emotive subscale were lower than those of the physical and partner-related subscales. The behavioral/emotive subscale examines sexual desire, sexual relationship frequency, and orgasm state of women. It was interesting that the scores in this subscale in the 4 groups were lower when compared with the other subscales. According to the literature search, the averages of the behavioral/emotive subscales in other studies that assessed sexual function using PISQ-12 or PISQ questionnaires were higher than the averages of the women in our study[22,24,25]. The considerably low averages of the behavioral/emotive subscales in different POP types and the lack of a difference between the groups gave rise to the thought that this situation may not be related to POP but may be related to the conservative perspective of Turkish society towards sexuality. It is thought that factors such as sexual appetite is regarded as wrong in Muslim societies and sexuality among women is regarded as a requirement for having children and making their husbands happy, which causes repressed sexuality[26].

It was previously reported that pelvic floor muscle strength, endurance, vaginal resting pressure, and pelvic floor muscle thickness decreased in women with POP[27]. To our knowledge, no studies have examined the effect of POP type on muscle strength. In this study, it was seen that pelvic floor muscle strength was similar between the groups. It was found that the sum of the muscle strength scores of the women with posterior compartment prolapse were higher than those of the apical and anterior compartment, and they were almost the same in asymptomatic women. This situation stems from the support of the posterior compartment by the PB and levator ani muscles, in addition to facial support[28].

The PFDI-20 is used frequently for the assessment of pelvic floor symptoms of women[2]. In our study, it was found that the total scores of pelvic floor distress, urinary, and colorectal-anal symptoms were similar. Prolapse symptoms in women with anterior and apical compartment POP were found more severe when compared with asymptomatic women. To the best of our knowledge, there are no studies in the literature investigating pelvic floor distress symptoms according to POP types. We think that the reason for the more severe prolapse distress symptoms among women with anterior and apical compartment prolapses, and the absence of a statistical difference between women with posterior compartment prolapse and asymptomatic women was the support of posterior structures with strong power such as the levator ani. In addition, the unequal and widely distributed numbers of patients included in this retrospective study may have affected the results. It is known that increased body weight is a risk factor for POP[29]. We anticipated that the BMIs of asymptomatic women would have been lower in this study, but there was no difference between the compartments. It was observed that the BMIs of women with apical compartment prolapses were lower than those of women with anterior compartment prolapses.

The strength of this study was the inclusion of only women with POP, the same race of women, and using a condition-specific questionnaire for sexual function. When it is considered that muscle strength could also affect sexual function, objective and the quantitative measurement of pelvic floor muscle strength of women was another strength of this research. Despite the conservative perspective of Turkey towards sexuality, questioning sexual function of women in a rural province was an extraordinary situation for a Muslim society.

Study Limitations

This research has a limitation due to its retrospective nature. It caused the exclusion of women who were sexually inactive because PISQ-12 is an appropriate questionnaire only for sexually active women. In 2013 the questionnaire was revised as the Pelvic Organ Prolapse/Incontinence Sexual Questionnaire, International Urogynecological Association-Revised (PISQ-IR), which also assesses sexually inactive women. However, the questionnaire could not be used because there was no Turkish version at the start date of the study. Another limitation of this study was the inclusion of only asymptomatic women with stage 1 prolapse as a control group and exclusion of women with stage 0 POP. Women with stage 0 POP were not accepted as the control group because they did not present to our women's health unit due to the lack of symptoms. Despite the presentation of women with stage 0 POP to our unit with pelvic floor dysfunction symptoms, this situation was the reason for our exclusion criterion. The lack of questioning sexual intercourse frequency, vaginal dryness, and body image perception are among our other limitations. It was reported in the literature that body image perception affected sexual function in particular, rather than POP's topographic changes[12].

Conclusion

This study illustrated that sexual function and muscle strength may not be affected by prolapse type. It was determined that there were more prolapse symptoms and complaints in women with anterior and apical compartment prolapses. When it is considered that POP can exist in more than one compartment simultaneously, more studies are needed including women with more than one compartment prolapse with larger samples. We think that investigating the effect of POP on sexual desire with the inclusion of sexually inactive women will be influential to determine the negative aspects caused by POP with the addition of PISQ-IR to the literature. Although there is no difference when comparing sexual function relative to compartments, low behavioral/emotive sub-domain scores of women may actually indicate problems with their orgasm, frequency of sexual intercourse, and sexual desire. For this reason, clinicians should examine women without discriminating compartments and women should be directed to appropriate treatment.

Ethics

Ethics Committee Approval: The study was approved by the Abant İzzet Baysal University Local Ethics Committee (approval number: 2014/17).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ö., Y.B., Concept: N.Ö., Y.B., Design: N.Ö., Y.B., Data Collection or Processing: N.Ö., Analysis or Interpretation: H.Ç., E.D., M.F.U., Literature Search: H.Ç., E.D., M.F.U., Writing: N.Ö., H.Ç., E.D., M.F.U., Y.B.

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Comparison of libido, Female Sexual Function Index, and Arizona scores in women who underwent laparoscopic or conventional abdominal hysterectomy

Laparoskopik veya konvansiyonel abdominal histerektomi yapılan kadınlarda libido, Kadın Seksüel Fonksiyon İndeksi ve Arizona skorlarının karşılaştırılması

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Abstract

Objective: The aim of the present study was to compare female sexual function between women who underwent conventional abdominal or laparoscopic hysterectomy.

Materials and Methods: Seventy-seven women who were scheduled to undergo hysterectomy without oophorectomy for benign gynecologic conditions were included in the study. The women were assigned to laparoscopic or open abdominal hysterectomy according to the surgeons preference. Women with endometriosis and symptomatic prolapsus were excluded. Female sexual function scores were obtained before and six months after the operation from each participant by using validated questionnaires.

Results: Pre- and postoperative scores of three different quationnaires were found as comparable in the group that underwent laparoscopic hysterectomy (p>0.05). Scores were also found as comparable in the group that underwent laparotomic hysterectomy (p>0.05). Pre- and postoperative values were compared between the two groups and revealed similar results with regard to all three scores (p>0.05).

Conclusion: Our data showed comparable pre- and the postoperative scores for the two different hysterectomy techniques. The two groups were also found to have similar pre- and postoperative score values.

Keywords: Sexual function, libido, hysterectomy, Female Sexual Function Index, Arizona Sexual Experiences Scale

Öz

Amaç: Bu çalışmanın amacı laparoskopik veya konvansiyonel abdominal histerektomi yapılan kadınlarda kadın seksüel fonksiyonlarının karşılaştırılmasıdır.

Gereç ve Yöntemler: Ooforektomisiz histerektomi yapılması planlanan 77 kadın çalışmaya dahil edildi. Cerrahın tercihine göre hastalar laparoskopik veya konvansiyonel abdominal histerektomi yapılmak üzere iki gruba randomize edildi. Endometriozis ve/veya semptomatik pelvik organ prolapsusu olan hastalar dışlandı. Kadın seksüel fonksiyon skorları ameliyat öncesinde ve 6 ay sonrasında valide edilmiş anketlerle sorgulandı.

Bulgular: Laparoskopik histerektomi yapılan grupta ameliyat öncesinde ve sonrasında yapılan skorlama sonuçları benzerdi (p>0,05). Skorlar laparotomik histerektomi yapılan grupta da benzer saptandı (p>0,05). İki grup arasında her üç anket için pre- and postoperatif skorlar benzerdi (p>0,05).

Sonuç: Çalışmamızda her iki histerektomi tipinde pre- ve postoperatif skorlar benzer saptandı. Gruplar içinde pre- ve postoperatif skorlar benzerdi.

Anahtar Kelimeler: Seksüel fonksiyon, libido, histerektomi, Kadın Seksüel Fonksiyon İndeksi, Arizona Seksüel Deneyimler Ölçeği

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PRECIS: Comparable pre- and the postoperative sexual function scores were obtained following two different hysterectomy techniques.

Introduction

Hysterectomy is a commonly performed surgical procedure[1]. Hysterectomy can be performed via the vaginal route or by applying minimally invasive techniques (laparoscopy, robotic surgery). Approximately 50% of cases undergo concomitant bilateral oophorectomy[2], as a consequence, estrogen deficiency may influence women's health. Furthermore, estrogen and androgen deficiency secondary to oophorectomy may aggrevate climacteric symptoms and sexual dysfunction, which may affect sexual pleasure, comfort, and frequency, resulting in lower desire, arousal, lubrication and sexual satisfaction. In addition, coital pain is a frequent sexual problem reported after perimenopausal oophorectomy[3].

Some validated and non-validated quastionnaires introduced the term "sexual function" as an overall descriptive term for outcomes[4] that include sexual activity and sexual function in terms of specific functional aspects, as well as satisfaction with sexual activity.

Age, menopausal status, systemic diseases, and also gynaecologic surgery were reported to adversely affect the sexual response[5]. It was reported that gynaecologic surgery may interfere with sexuality in middle-age women and some factors played a significant role leading to dysfunction, including changing self-image, sexual pain and orgasm difficulty[6]. Sexual wellbeing may differ according to the type of hysterectomy because different techniques damage the innervation and supportive structures of the pelvic floor. Recent technical advances made laparoscopic surgery possible in many surgical fields and laparoscopy became a feasable technique for hysterectomy[7].

In this study, we aimed to assess the effect of laparotomic versus laparoscopic hysterectomy techniques on the Female Sexual Function Index (FSFI), the Libido Scoring System (LSS), and Arizona scores.

Materials and Methods

Participants

This prospective observational study was performed at the Gynecology Clinic of Zeynep Kamil Women and Children's Health Training and Reseach Hospital between June 2014 and December 2015. Informed consent was obtained from each participant.

Seventy-seven consecutive women who were sexually active and healthy premenopausal patients, aged between 40-55 years, and were offered hysterectomy for benign indications either via laparotomy or laparoscopy were included in the study. The technique of hysterectomy was determined according to the surgeon's preference. Exclusion criteria consisted of endometriosis, symptomatic prolapsus, chronic pelvic pain and malignancy as indications for surgery, patients with sexual dysfunction, participation refusal or reduced capability of understanding the survey, patients with severe depression or had been using antidepressant treatment, and patients whose partner had a severe illness or had died recently. Patients who required oophorectomy during the operation (n=3), developed complications in the postoperative period (n=2), and those who refused to participate in the study after the operation (n=6) were excluded.

The FSFI, Arizona, and LSS questionnaires were completed in face-to-face sessions. Sociodemographic data (personal and partner) were recorded. In total, 66 patients completed these questionnaires for evaluating sexual function prior to and six months after hysterectomy. Hysterectomies were performed by the same surgical team according to standard surgical techniques.

The Female Sexual Function Index

The FSFI is a validated self-administered questionnaire that consists of 19 questions and measures six domains of sexual function: desire, arousal, lubrication, orgasm, satisfaction and pain[8]. The first and second questions have scales from 1 to 5 and other questions have scales from 0 to 5 for scoring. Scores obtained in a particular domain are added and multiplied by a respective factor (coefficients for questions 1-2: 0.6; 3-10: 0.3; 11-19: 0.4), which homogenizes the influence of each dimension. A total sum of each score is obtained and higher scores indicate healthy sexual life. Score ranges between 1.2 and 36. An optimal cut-off was introduced as 26[9]. In the present study, an FSFI score of 26 or less was defined as sexual dysfunction. Validation of this questionnaire in Turkish population was shown in previous reports[10].

The Arizona Sexual Experiences Scale

The Arizona Sexual Experiences Scale (ASEX) has five items that assess sexual experiences including: drive, arousal, vaginal lubrication, ability to reach orgasm, and satisfaction with orgasm. The lubrication item is assessed by the versions specific for sex. Each item is rated with a six-point scale. Scores range between 5 and 30; higher scores indicate better sexual life. Use of this questionnaire was shown in previous reports[11].

The Libido Scoring System

LSS was developed in 1997 by Api et al.[12]. It comprises four questions on four domains: orgasmic function, coital frequency, sexual desire, and sexual self-interest (masturbation). FSFI was well-correlated with the LSS, revealing a correlation coefficient of 0.96 ($p<0.001$) (the Cronbach's α coefficient was found as 0.83 and the total kappa values were 0.67 and 0.77)[12]. The patients were scored and a total score less than 3 was considered as loss of libido; scores of 3 and 4 were considered as low libido,

5-7 as moderate libido, and 8-12 as high libido. The researcher asked the following questions to the patients:
1. How often do you have sex or masturbate?
2. Do you masturbate?
3. Who starts the sexual activity? (Who asks for or implies sex first?)
4. Do you have orgasm during masturbation or sexual intercourse?

Statistical Analysis

Data were analyzed using SPSS 15.0 for Windows. Student's t-test was used to compare continuous variables between the groups. The paired samples t-test was used to show comparisons of continuous variables before and after intervention. P values <0.05 were accepted as statistically significant.

Results

The groups were similar with regard to their mean age (44 vs. 46 years) and mean uterine volume (730 vs. 1050 cm^3, p>0.05). The pre- and postoperative scores of the three different quationnaires were found as comparable in the group that underwent laparoscopic hysterectomy (p>0.05, Table 1). Scores were also found as comparable in the group that underwent laparotomic hysterectomy (p>0.05, Table 2). Pre- and postoperative values were compared between the two groups, which revealed similar results with regards to all three scores (p>0.05, Table 3).

Discussion

In this study, we compared the pre- and the postoperative sexual function of women who underwent laparoscopic or laparotomic hysterectomy. Our data revealed comparable pre- and postoperative scores between the groups. The comparison of pre- and postoperative score values within each group revealed similar results. We preferred to assess sexual function at the 6th postoperative month based on a study that indicated a requirement of 6 months for pelvic innervation recovery[13]. Hysterectomy is a frequently performed gynecologic surgery for variable indications. Sexual function after hysterectomy with different techniques has been questioned in several trials and generally it was thought that injury to the uterovaginal plexus during hysterectomy might interfere with the neuronal support of vagina, which leads to affected orgasm and lubrication[14,15]. There are also some data indicating similar sexual function in women with and without cervical ablation[16,17], and studies also showed similar sexual function among different hysterectomy techniques including total abdominal hysterectomy, subtotal hysterectomy, and vaginal hysterectomy[18].

A recently published review on this issue assessed the results of previously published studies and summarized the results under different headings including sexual desire, sexual arousal,

Table 1. Comparison of pre- and postoperative scores of three different sexual function evaluation questionnaires in women who underwent laparoscopic hysterectomy

	Mean	n	Standard deviation	p
Preoperative libido	3.6	35	1.5	>0.05
Postoperative libido	3.6	35	1.5	
Preoperative FSFI	21.6	35	7.8	>0.05
Postoperative FSFI	23.6	35	6.4	
Preoperative Arizona	16.3	35	5.2	>0.05
Postoperative Arizona	15.9	35	4.5	

FSFI: Female Sexual Function Index

Table 2. Comparison of pre- and postoperative scores of three different sexual function evaluation questionnaires in women who underwent laparotomic hysterectomy

	Mean	n	Standard deviation	p
Preoperative libido	3.6	31	1.7	
Postoperative libido	3.9	31	1.6	>0.05
Preoperative FSFI	21.7	31	7.8	
Postoperative FSFI	23.2	31	5.6	>0.05
Preoperative Arizona	17.4	31	6.9	
Postoperative Arizona	15.2	31	4.4	>0.05

FSFI: Female Sexual Function Index

Table 3. Comparison of three different sexual function evaluation questionnaire scores between women underwent laparoscopic and laparotomic hysterectomy

	Groups	n	Mean	Standard deviation	p
Preoperative libido	LS	35	3.6	1.5	
	LPT	31	3.6	1.7	>0.05
Postoperative libido	LS	35	3.6	1.4	
	LPT	31	3.9	1.6	>0.05
Preoperative FSFI	LS	35	21.6	7.7	
	LPT	31	21.7	7.1	>0.05
Postoperative FSFI	LS	35	23.6	6.4	
	LPT	31	23.2	5.6	>0.05
Preoperative Arizona	LS	35	16.2	5.1	
	LPT	31	17.4	6.9	>0.05
Postoperative Arizona	LS	35	15.8	4.4	
	LPT	31	15.2	4.4	>0.05
Uterine volume	LS	35	730.9	1321.6	
	LPT	31	1050.2	1007.3	>0.05

LS: Laparoscopy, LPT: Laparotomy, FSFI: Female Sexual Function Index

orgasm, dyspareunia and sexual satisfaction, and the authors reported contradictory results with regard to these sexual parameters[19]. All these parameters were assessed by using three different questionnaires in our study. We found similar pre- and postoperative scores for each technique.

In contrast to our results, some studies in the literature showed measurable advances in life style and sexual function after simple hysterectomy, whereas others revealed negative results[20-23]. Furthermore, a previous study indicated that hysterectomy contributed to quality of life with minimal postoperative morbidity after minimally invasive surgery[24].

Consistent with our results, according to some prospective, randomized studies, late psychosexual changes have not been thought to primarily depend on the surgical method (vaginal, abdominal total or subtotal hysterectomy); studies showed the quality of sexual relationship before the operation as the most significant predictive factor[25].

The aforementioned conclusions show several conflicting results with regard to the impact of hysterectomy on sexual function. Some authors suggested that the parameters of questionnaires were unsatisfactory for assessing sexual function[26].

Meta-analyses on this issue revealed that prolapsus operations, particularly posterior repairs using levator plication, seemed to deteriorate sexual function, and hysterectomy was found to improve sexual function, regardless of whether it was subtotal or total. The review concluded that gynecologic operations might influence sexual function; however, little validated data are available to come to this conclusion[27]. Due to the lack of validated data, we performed this study by using three different scoring systems including ASEX, which was shown to be a valid and reliable instrument for use in clinical trials on sexual function in the Turkish population[28], and FSFI scoring, which has been used in several different studies in the Turkish population; validation of this scoring system for the Turkish population has been shown[29,30]. Finally, we also tried to confirm our results by the third different scoring system introduced by the Api et al.[12] who concluded that this simple test provided a reliable measure for routine clinical practice or trial purposes.

Most of the studies in the literature included heterogeneous groups of participants to assess sexual function and the majority failed to exclude patients with certain factors (e.g., menopausal status, comorbidity, oophorectomy, endometriosis, malignity), which may interfere with the results[31]. Our data originated from a homogeneous group of patients from a single tertiary referral center; most women with the aforementioned factors that may be interfere with the results were excluded from our study and we used three different questionnaires comprising different parameters to assess the effect of surgical technique on sexual function.

Study Limitations

Small sample size is the major drawback in our study.

Conclusion

Our data showed comparable pre- and the postoperative scores for the two different hysterectomy techniques. Pre- and postoperative scores were similar within each surgical technique, using three different questionnaires revealed no effect of surgical technique on sexual function after hysterectomy.

Ethics

Ethics Committee Approval: Ethics committee approval was not obtained because of the observational study.

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Clinical Investigation / Araştırma

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Features of ovarian Brenner tumors: Experience of a single tertiary center

Ovaryan Brenner tümörlerinin özellikleri: Üçüncü basamak merkez tecrübesi

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Abstract

Objective: Brenner tumors are rare neoplasms of the ovary. The aim of this study was to investigate the clinical features of Brenner tumors.

Materials and Methods: The clinical features of 22 patients who were treated in Ankara University Faculty of Medicine Obstetrics and Gynecology Department between 2005 and 2015 were evaluated retrospectively from hospital medical records.

Results: The patients were aged 34 to 79 years at the time of diagnosis and the mean age was 55.1 years. Two (9.1%) patients were pre-menopausal, five (22.7%) were peri-menopausal, and 25 (68.2%) patients were postmenopausal. One patient was pregnant. Twenty of the neoplasms were benign, one was malignant, and one was both malignant and benign. There was no recurrence in the malignant cases. Six (27.2%) patients had mixed tumors consisting of Brenner tumor and another ovarian pathology. Specifically, the other component of these tumors was mucinous cystadenoma in four patients, endometriosis externa in one patient, and high-grade serous papillary cyst adenocarcinoma in one patient.

Conclusion: Brenner tumors are usually incidental benign pathologic findings of surgical procedures in postmenopausal women. They can be found with other ovarian pathologies such as mucinous ovarian tumors and can coexist with other female genital tumors. Further studies are needed to completely understand the clinical features of Brenner tumors.

Keywords: Brenner tumor, ovarian neoplasm, gynecologic oncology

Öz

Amaç: Brenner tümörleri overin nadir neoplazmlarındandır. Bu çalışmanın amacı Brenner tümörlerinin klinik özelliklerinin incelenmesidir.

Gereç ve Yöntemler: Ankara Üniversitesi Tıp Fakültesi Obstetrik ve Jinekoloji Anabilim Dalı'nda tedavi alan 2005-2015 yılları arasındaki 22 hasta retrospektif olarak incelenmiştir.

Bulgular: Hastaların tanı aldıklarında ortalama yaşı 55,1 olmakla beraber yaş aralığı 34 ile 79 arasındaydı. İki hasta (%9,1) pre-menapozal dönemdeyken, beş hasta (%22,7) peri-menapozal dönemde ve on beş hasta (%68,2) postmenapozal dönemdeydi. Hastaların bir tanesi tanı sırasında gebeydi. Yirmi hastanın final patolojisi benign Brenner neoplazmı iken, bir tanesi malign ve bir tanesi hem benign hem malign olarak raporlanmıştır. Malign hastalarda takip süresince rekürrens görülmedi. Altı hastada (%27,2) Brenner tümörüyle beraber seyreden başka bir ovaryan patoloji vardı. Bu hastaların dördünde eşlik eden patoloji müsinöz kistadenoma iken bir hastada endometriyozis eksterna ve bir hastada yüksek gradeli seröz papiller kist adenokarsinom izlendi.

Sonuç: Brenner tümörleri, genellikle postmenapozal dönemdeki kadınlarda cerrahi prosedürler sonrası rastlantısal olarak bulunan benign patolojik bulgulardır. Özellikle müsinöz over tümörü gibi başka bir ovaryan patolojiye eşlik edebilirler. Diğer kadın genital tümörleri ile beraber görülebilirler. Brenner tümörlerinin klinik özelliklerinin tam olarak anlaşılabilmesi için ek çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Brenner tümörü, ovaryan neoplazm, jinekolojik onkoloji

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PRECIS: We have investigated the clinical features of rare Brenner tumors with twenty two cases were operated in our clinic.

Introduction

Brenner tumors are a relatively rare surface epithelial neoplasm of the ovary, accounting for 1.4-2.5% of all ovarian tumors. They are known as transitional cell tumors of the ovaries due to their histologic findings. Brenner tumors usually affect postmenopausal women, and most (99%) are benign[1-4]. They are usually unilateral; bilateral lesions are found in 5-14% of cases[2,4,5]. Although Brenner tumors are usually discovered incidentally, patients occasionally present with symptoms such as a palpable mass or pain[6]. Here, we present the clinical findings of Brenner tumors that were diagnosed and treated between 2005 and 2015 in Ankara University, Department of Obstetrics and Gynecology.

Materials and Methods

Patients with a histologic diagnosis of Brenner tumor of the ovary who underwent laparotomic or laparoscopic surgery for any reason between 2005 and 2015 were identified from the database of Ankara University's Faculty of Medicine Department of Obstetrics and Gynecology. The records of 22 patients were diagnosed as having Brenner tumors were retrieved. Data on age, clinical findings, menopausal status, tumor size, surgical procedure, associated pathologic findings, side of the tumor, and clinical follow-up period were collected. Follow-up information was taken retrospectively from the medical records of patients' last visits of our hospital. In this study, all data were assessed retrospectively and neither ethics committee approval nor patients' informed consents were obtained. This study was reviewed by the appropriate ethics committee and was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 20.0 software (IBM Corporation Software Group, New York, United States of America).

Results

Twenty two cases of Brenner tumor were diagnosed over a ten-year period. The clinical characteristics are summarized in Table 1. The patients were aged 34 to 79 years at the time of diagnosis and the mean age was 55.1 years. Two (9.1%) patients were pre-menopausal, five (22.7%) were peri-menopausal, and 15 (68.2%) were postmenopausal. One patient was pregnant.

Eight (36.3%) patients were admitted to our department because of adnexal mass and their main symptom was abdominal pain. The other patients' main symptoms were divergent. Seven (31.8%) patients presented with postmenopausal bleeding, two (9.1%) with menometrorrhagia, two (9.1%) with uterine descensus, one (4.55%) with ascites, and one (4.55%) with vaginal bleeding and a vaginal mass. Among the patients, only two had clinical symptoms caused by Brenner tumors and these were malignant.

The left ovary was involved in 12 cases, the right in 8 cases, and bilateral Brenner tumors existed in 2 cases (9.1%). The smallest tumor had a maximum diameter of 2 mm and the largest tumor a maximum diameter of 20 cm. Twenty of the neoplasms were benign, one was malignant, and one was both malignant and benign.

Five (22%) patients underwent surgery for endometrial malignancy and one for cervical malignancy. Nine patients presented with adnexal mass or ascites. Among these patients, the final pathology of two patients was benign pure Brenner tumor. Two cases (9.1%) were diagnosed as malignant Brenner tumor, and one as high-grade serous papillary cyst adenocarcinoma. Four cases (18.2%) were mixed tumors consisting of Brenner tumor and another ovarian pathology. Specifically, the other component of these tumors was mucinous cystadenoma. One patient was admitted with uterine descensus and their final pathology was coexistence of endometriosis externa and Brenner tumor. One cesarean section was performed for the previous cesarean delivery indication and a benign Brenner tumor was diagnosed incidentally.

Case 10 had a bilateral malignant Brenner tumor (stage III C) and she underwent total abdominal hysterectomy with bilateral salpingo-oopherectomy, ommentectomy, bilateral pelvic paraaortic lymphadenectomy, splenectomy, and appendectomy. The preoperative CA-125 was 51 U/mL. The patient received six cycles of carboplatin-paclitaxel chemotherapy and has been in follow-up for 2 years with no recurrence. The final pathology of case 11 was grade 2, stage III C malignant Brenner tumor in the right ovary, and a benign tumor in the left ovary. The patient received the same chemotherapy protocol as case 10 and there was no recurrence after surgery during 18 months of follow-up.

Fifteen patients' tumor marker CA-125 levels were known before surgery. Levels of CA-125 were high in only two patients; they were normal in the remaining patients (CA-125 cut-off level 35 IU/mL).

Discussion

Brenner tumors are usually benign tumors, although there is a wide spectrum between benign and malignant features[7]. In contrast to the benign tumors, malignant Brenner tumor cells have hyperchromatic, pleomorphic nuclei and numerous mitotic figures, and they are characterized by destructive stromal invasion[8,9]. In our case series, 20 (90.9%) patients had benign Brenner tumors and two (9.1%) patients had malignant Brenner tumors (stage III C). Approximately 1% of Brenner tumors in the literature were determined as malignant[3,10]. Our results are inconsistent with the literature, but this might be

Table 1. Clinical characteristics of the patients

Case	Age-years	Menopause	Clinical findings	Side	Tumor size	Preoperative CA-125 level (U/mL)	Surgical procedure	Associated condition
1	48	No	Menometrorrhagia	L	18 mm	12.3	TAH, BSO	Leiomyoma
2	50	No	Menometrorrhagia	L	2 mm	-	TAH, BSO	Endometrial polyp, leiomyoma
3	55	Yes	Desensus uteri	R	10 mm	-	TAH, BSO	Leiomyoma
4	34	No	Pregnancy	R	4.5 cm	-	C/S, right ovarian cyst excision	-
5	49	No	Vaginal bleeding, vaginal mass	L	14 mm	13.5	TAH, BSO	Leiomyoma
6	62	Yes	Postmenopausal bleeding	R	2 mm	10	TAH, BSO, BPPLND, ommentectomy	Endometrial cancer stage IB grade II
7	59	Yes	Postmenopausal bleeding	R	3 cm	-	TAH, BSO	Endometrial polyp, leiomyoma
8	58	Yes	Abdominal pain, adnexal mass	L	20 cm	8.4	TAH, BSO, BPLND, ommentectomy, appendectomy	Leiomyoma, mucinous cystadenoma
9	57	Yes	Postmenopausal bleeding	L	1.5 cm	32.2	TAH, BSO, BPPLND, ommentectomy	Endometrial cancer stage III C grade III
10	49	Yes	Abdominal pain, adnexal mass	L, R	14 cm	51	TAH, BSO, BPPLND, ommentectomy, appendectomy, splenectomy	High-grade malignant Brenner tumor stage IIIC
11	62	Yes	Abdominal pain, adnexal mass	L, R	18 mm	24	TAH, BSO, BPPLND, ommentectomy, appendectomy	Grade II malignant Brenner tumor, leiomyoma
12	78	Yes	Abdominal pain, ascites	L	3.5 cm	152	BSO, BPPLND, ommentectomy, splenectomy	High-grade serous papillary cyst adenocarcinoma stage III C
13	60	Yes	Postmenopausal bleeding	R	3 cm	14	TAH, BSO, BPPLND, ommentectomy, appendectomy	Cervical cancer stage I C grade II
14	79	Yes	Adnexal mass	L	16 cm	-	Laparoscopic USO	-
15	47	No	Adnexal mass	R	8 cm	14.2	TAH, BSO	-
16	24	No	Abdominal pain, adnexal mass	L	6 cm	9.4	Laparoscopic ovarian cyst excision	Mucinous cystadenoma
17	58	Yes	Adnexal mass	L	5 cm	8.4	TAH, BSO, BPLND, ommentectomy, appendectomy	Mucinous cystadenoma

Table 1. Clinical characteristics of the patients

18	57	Yes	Postmenopausal bleeding	L	3 cm	-	TAH, BSO	Endometrial cancer stage I A grade II
19	53	Yes	Postmenopausal bleeding	R	9 mm	12	TAH, BSO, BPPLND, ommentectomy, appendectomy	Endometrial cancer stage I A grade II
20	71	Yes	Abdominal pain, adnexal mass	L	11 cm	5	TAH, BSO, ommentectomy	Mucinous cystadenoma
21	49	No	Desensus uteri	R	1 cm	-	TAH, BSO	Leiomyoma, endometriosis externa
22	74	Yes	Postmenopausal bleeding	L	1.5 cm	10	TAH, BSO, BPLND, ommentectomy, appendectomy	Endometrial cancer stage I B grade II

TAH: Total abdominal hysterectomy, BSO: Bilateral salpingo-oopherectomy, *CS*: Cesarean section, BPPLND: Bilateral pelvic and paraaortic lymph node dissection, BPLND: Bilateral pelvic lymph node dissection, USO: Unilateral salpingo-oopherectomy, R: Right, L: Left

because our clinic is a tertiary referral oncology center and the proportion of malignancy is higher than in the normal population.

Brenner tumors have a predilection for postmenopausal women in the literature[3,11,12]. In our study, the mean age of the patients was 55.1 years and 68.2% of the patients were postmenopausal, in accordance with the literature. Four patients (18.1%) had a mucinous cystadenoma. Coexistence of mucinous cystadenoma and Brenner tumor is consistent with the literature[13]. It is important that 25% of all mucinous ovarian tumors have a minor Brenner tumor component[14]. In our study, seven (33.3%) patients had postmenopausal bleeding and the final pathology of five patients was reported as endometrial carcinoma. Also, three patients presented with abnormal vaginal bleeding. In one previous study, the authors reported that histologically confirmed endometrial hyperplasia coexisted in 4-14% of women who had Brenner tumors[15]. Additionally, several cases were reported as having abnormal vaginal bleeding and endometrial hyperplasia in another study[16]. Synchronous multiple primary tumors of the female genital tract account for only 1-6% of all genital neoplasms. In the literature, the coexistence of endometrial cancer and ovarian cancer is the most frequently observed synchronous tumor occurrence, like in our study[17,18]. There are no data about the coexistence of cervical cancer and Brenner tumors in the literature; one (4.5%) patient had a grade 2 cervical cancer in our study.

Diagnosing Brenner tumors with imaging studies is difficult because the tumor's appearance is nonspecific[19,20]. Therefore, before surgery, we could not estimate that Brenner tumors were present for any of the patients in our study. Although Brenner tumors are usually discovered incidentally, they sometimes have symptoms such as a palpable mass or pain[6]. Two patients presented with abdominal pain due to a Brenner tumor and there was no other pathologic finding for pain. These two cases were reported as malignant Brenner tumors and no benign Brenner tumors were symptomatic at the time of diagnosis.

Usually, benign Brenner tumors are unilateral and malignant Brenner tumors are bilateral[21]. In our study, one malignant tumor was bilateral and the other was unilateral. There was a benign Brenner tumor contralaterally in the unilateral malignant tumor case. All benign Brenner tumors were unilateral. Dierickx et al.[20] and Blaustein's[8] textbook of pathology reported that prevelance of unilateral lesion was higher in the left ovary[9]. In our study, unilateral Brenner tumors were more common in left ovaries, in accordance with the literature.

Some authors indicated that malignant Brenner tumors had better prognosis than other epithelial ovarian tumors[7]. In our cases, there was no recurrence but this could be explained by the short follow-up period and the small number of malignant tumors.

The treatment for Brenner tumor is essentially surgical. Surgical staging should be done if a tumor has malignant potential. The role of lymphadenectomy is not yet clear because of the rare

occurence of malignant Brenner tumors. It is reported that the rate of lymph node metastasis was 5.1% and lymphadenectomy was not associated with any improvement in survival[22]. Nasioudis et al.[22] and Han et al.[23] reported that the majority of malignant Brenner tumors presented with localized disease (stage I). In contrast to these published articles, the two patients with malignant tumors in our study presented at stage III, consistent with Gezginç et al.[24].

Study Limitations

In this study, data were retrieved retrospectively, which is the limitation of our study.

Conclusion

Brenner tumors are usually benign neoplasms of the ovary, which are frequently diagnosed incidentally during surgical procedures. They are mostly seen in the postmenopausal period with vaginal bleeding and can coexist with other ovarian pathologies and female genital tumors.

Brenner tumors require further intervention trials and studies because of the limited number of trials.

Ethics

Ethics Committee Approval: Ethics commitee approval was not obtained because of retrospective design.

Informed Consent: Informed consent form was not obtained because of retrospective design.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: B.T., S.T., U.F.O., Design: B.T., S.T., U.F.O., Data Collection or Processing: B.T., K.K., Analysis or Interpretation: B.T., K.K., Literature Search: K.K., Writing: B.T.

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Case Report / Olgu Sunumu

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Harlequin ichthyosis: A rare case

Harlequin iktiyozis: Nadir bir durum

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Abstract

Harlequin ichthyosis is a very rare condition that affects the skin of newborns. It is associated with poor barrier function of the skin leading to dehydration and leaves newborns prone to infections. It is due to mutations in adenosine triphosphate binding cassette *A12* gene transmitted as an autosomal recessive disorder. The prognosis is very poor in these cases. Here, we report one such rare case.
Keywords: Harlequin ichthyosis, adenosine triphosphate binding cassette A12, autosomal recessive

Öz

Harlequin iktiyozis yenidoğanların cildini etkileyen nadir bir durumdur. Dehidrasyona sebep olan cildin zayıf bariyer fonksiyonuyla ilişkilidir ve yenidoğanın enfeksiyona yatkın olmasına sebep olur. Adenozin trifosfat bağlayıcı kaset *A12* genindeki mutasyonların otozomal resesif hastalık olarak iletilmesi sebebiyle oluşur. Bu olgular için tanı oldukça zayıftır. Bu durumdaki bir olguyu sunuyoruz.
Anahtar Kelimeler: Harlequin iktiyozis, adenozin trifosfat bağlayıcı kaset A12, otozomal resesif

Introduction

Ichthyosis is derived from a Greek word, ichthys, which means fish. It refers to a fish-scale-like appearance of skin. Ichthyoses are disorders of skin characterized by dry, scaly and thickened skin. Ichthyosis may involve the skin alone or other organs also, and may be inherited or acquired. The mode of inheritance is autosomal or X-linked, and can be dominant or recessive. The 3 major types of autosomal recessive congenital ichthyosis are Harlequin ichthyosis, lamellar ichthyosis, and congenital ichthyosiform erythroderma. Harlequin ichthyosis is the least common and most severe form.

The first case was reported in South Carolina, United States of America, in 1750 by Hart[1]. The first case diagnosed antenatally was reported in 1983[2]. Antenatal diagnosis in suspected cases can be confirmed using electron microscopy of fetal skin biopsy and DNA-based diagnosis with chorionic villus sampling or amniocentesis[3]. There is no cure for this condition and only supportive treatment can be given to prolong life.

Case Report

A primigravida woman aged 20 years registered with a private practitioner, reported to the labor room with 33 weeks' of gestation with preterm premature rupture of membranes in latent labor with breech presentation. A history of 2nd degree consanguinity was noted with 9 months of married life. An earlier scan detected polyhydramnios. The other abnormalities were not appreciated on the scan. She underwent emergency cesarean section in view of footling presentation and a female baby weighing 1.9 kg was delivered on December 22nd, 2016.

The baby had white porcelain-like skin covering the body like armor with deep creases all over the body as shown in Figure 1. Bleeding was noticed from the creases. The baby had a weak cry at birth. Eyelids and lips were everted showing ectropion and eclabion, respectively. Nasal hypoplasia with two nostrils was seen. The mouth was open with thick lips as seen in Figures 2 and 3. The ears were small with closed pinna. The fingers and toes were flexed and fixed flexion deformity noticed, as seen in Figure 4. The heart rate and respiratory rate were normal. The baby was sent to the neonatal intensive care unit for further management.

Later, the baby's skin was noticed to peel off leaving erythematous fissures. A peripheral intravenous line could not be secured and the umbilical vein was accessed. Conservative management was given with intravenous antibiotics, emollients, and retinoids. Feeds were given through a Ryles tube. The baby died on the

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3rd day. The parents refused an autopsy and skin biopsy of the baby.

The parents were called for genetic counseling.

Figure 1. Skin of the baby at birth showing armor-like covering with creases

Figure 2. Bleeding from the creases with ectropion and thick lips with mouth open

Discussion

Harlequin ichthyosis is also known as Harlequin baby syndrome/Harlequin fetus syndrome/ichthyosis congenita. The word Harlequin is derived from a similar appearance of a comic servant character. Harlequin ichthyosis is a rare form of congenital ichthyosis with an overall incidence of 1 in 300,000 births[3]. Approximately 200 cases have been reported throughout the world[4]. Recently, one case was reported in June 2016 at Nagpur, India, published in the International Journal of Pharmacy[5]. It is inherited as an autosomal recessive condition[3]. It can affect male and female children equally. It occurs due to a mutation in the adenosine triphosphate binding cassette *A12 (ABCA12)* gene, which is responsible for the exocytosis of lipid-containing lamellar granules, which control the process of desquamation[6]. The locus for the *ABCA12* gene lies on chromosome 2q35[7]. The major types of mutations responsible for this are nonsense mutations or frameshift mutations[7]. This condition can be diagnosed antenatally by scanning with the following features: polyhydramnios (seen in this case), echogenic amniotic fluid, fetal growth restriction, eyes closed with eversion of the eyelids and lips (ectropion and eclabion, respectively), flat nose, mouth wide open, ears not well formed, flexion of extremities, mottled, breeched skin of the face and limbs, hyperflexion of fingers and toes, absence

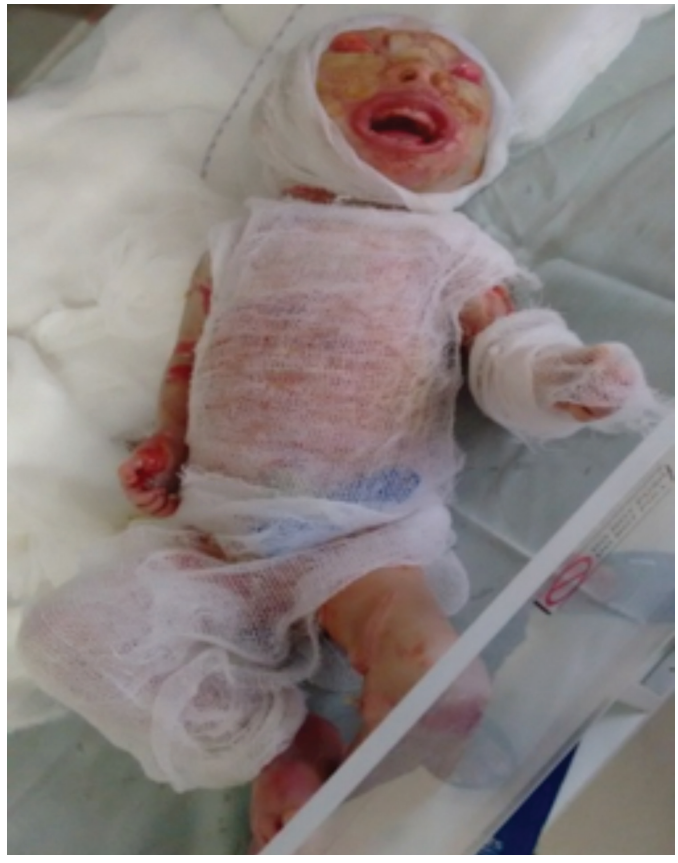

Figure 3. Ectropion, open mouth and thick lips seen. Emollients applied and the baby covered with sterile gauze

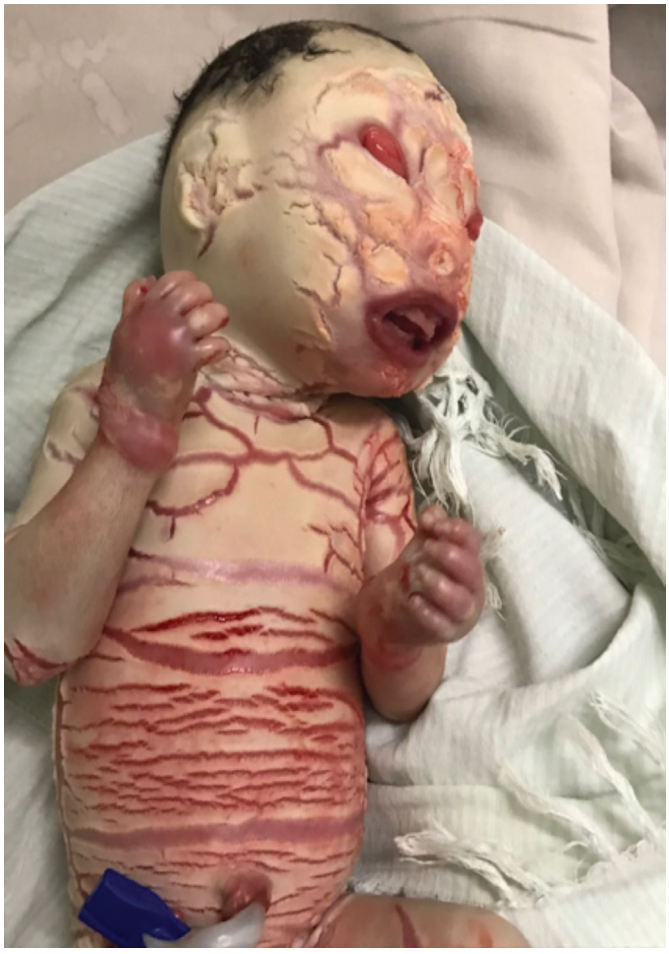

Figure 4. Ears are small with closed pinna. Fingers showing flexion deformity

of opening movements of fingers[1]. A 3D scan is better for diagnosing this condition.

Life-threatening complications include dehydration, supervening infections, and respiratory insufficiency. The prognosis is very poor. Most affected babies do not survive beyond the first week of life. It has been reported that the survival rate varies from 10 months to 25 years with supportive treatment depending on the severity of the condition[8]. Recurrence of this condition in the next pregnancy is 25%[1]. Genetic counseling should be undertaken for these cases.

Ethics

Informed Consent: Consent was given by the parents.
Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Case Report / Olgu Sunumu

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Early vulvar and umbilical incisional scar recurrence of cervical squamous cell carcinoma: Earlier than usually expected

Skuamöz hücreli servikal karsinomun erken vulvar ve umbilikal insizyonel skar rekürrensi: Beklenilenden daha erken

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Abstract

Cutaneous metastasis is considered as a hazardous condition depending on the mean survival around 9 months, which usually originates from cancers of the breast, lung, ovary, colon, and rarely from the cervix. The crucial prognostic factor of cutaneous metastasis depends on the period between the primary malignancy and cutaneous metastasis. We report two cases of the unusual presentation of squamous cell cancer of the cervix that developed vulvar and umbilical metastasis in the 5th month of primary treatment. Both of our patients survived for 11 months following the primary treatment. In addition, our first case is the earliest vulvar recurrence of cervical carcinoma in the English literature following appropriate medical and surgical management.
Keywords: Squamous cell cervical cancer, umbilical metastasis, vulvar metastasis

Öz

Deri metastazı ortalama sağkalım süresi 9 ay olan, genellikle meme, akciğer, over, kolon ve nadiren de serviksten köken alan tehlikeli bir durumdur. Deri metastazı ve primer malignensi arasındaki zaman deri metastazının oluşumunda çok önemli bir prognostik faktördür. Primer tedavinin 5. ayında gelişen skuamöz hücreli kanserin çok nadir görülen vulvar ve umbilikal metastaz olgularından ikisini sunuyoruz. Olgularımızın ikisi de primer tedavi sonrası 11 ay yaşadılar. Sunmuş olduğumuz ilk hasta uygun medikal ve cerrahi sonrasında serviks kanserinin İngilizce literatürdeki en erken vulvar rekürrensi olan olgudur.
Anahtar Kelimeler: Skuamöz hücreli serviks kanseri, umbilikal metastaz, vulvar metastaz

Introduction

Cervical cancer recurrence depends on the cancer's clinical stage and may manifest as local or distant metastasis in different organs. Recurrence occurs most commonly in the pelvis, which includes the parametrium or lymph nodes, and in the vagina. Recurrence can rarely occur in the skin, ranging between 0.1-1.3%. In most cases, they manifest as an asymptomatic dermal/subcutaneous plaque, ulcer or nodule[(1)].

We report two unusual presentations of cervical squamous cell carcinoma with early vulvar and umbilical metastasis.

Case Reports

Case 1

A woman aged 41 years was admitted to a state hospital with pelvic pain, urinary burning, and vaginal bleeding. The patient was referred to our hospital after a cervical biopsy revealed cervical squamous cell carcinoma. On our physical examination, we observed an exophytic necrotic mass measuring 8x9 cm confined to the cervix with no parametrial invasion. According to the International Federation of Gynecology and Obstetrics classification, we established the diagnosis as stage 1b-2

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cervical cancer. We excised the mass through the vagina route and performed a type 3 radical hysterectomy (Wertheim) with pelvic-paraaortic lymph node dissection followed by radiotherapy. In the fifth month after surgical treatment, we observed a 2x3-cm ulcerated nodular vulvar lesion (Figure 1). The lesion in the vulva was excised following abdominopelvic computerized tomography (CT) imaging, which revealed no significant pathology. The biopsy specimen showed squamous cell carcinoma (Figure 2). A paclitaxel and carboplatin combined chemotherapy protocol was used. Following 2 cycles of chemotherapy, positron emission tomography-CT revealed diffuse metastases in the abdominopelvic site. Despite and alternative protocol (gemcitabine and bevacizumab) administration, there was no response. The patient died in the 11th month of the postoperative period.

Case 2

A woman aged 54 years who was post-menopausal presented with vaginal bleeding that had persisted for 3 months. A vaginal examination revealed a cervical mass measuring 1x1.5 cm. Histologic examination of the mass showed cervical squamous cell carcinoma. A Wertheim operation was performed and there was no lymph node involvement and the mass had negative surgical borders (stage 1b-1). A Papanicolaou smear was obtained from the vaginal cuff 3 months later and the result was negative. The patient presented with severe abdominal pain, which was localized along the incisional scar region of her umbilicus five months after the primary surgical treatment. Abdominal CT revealed an umbilical mass measuring 4x4.5 cm in diameter (Figure 3). We considered that the fixed mass was inoperable; it included all layers of the umbilical wall and extended from umbilicus to the upper anterior abdominal wall with massive adhesions. We performed a partial resection of the mass and pathologic examination revealed metastatic squamous cell carcinoma. Two cycles of chemotherapy, including paclitaxel-carboplatin in the first cycle and bevacizumab-gemcitabine in the second cycle, and radiotherapy was administered. The patient died in the 11th month of her medication.

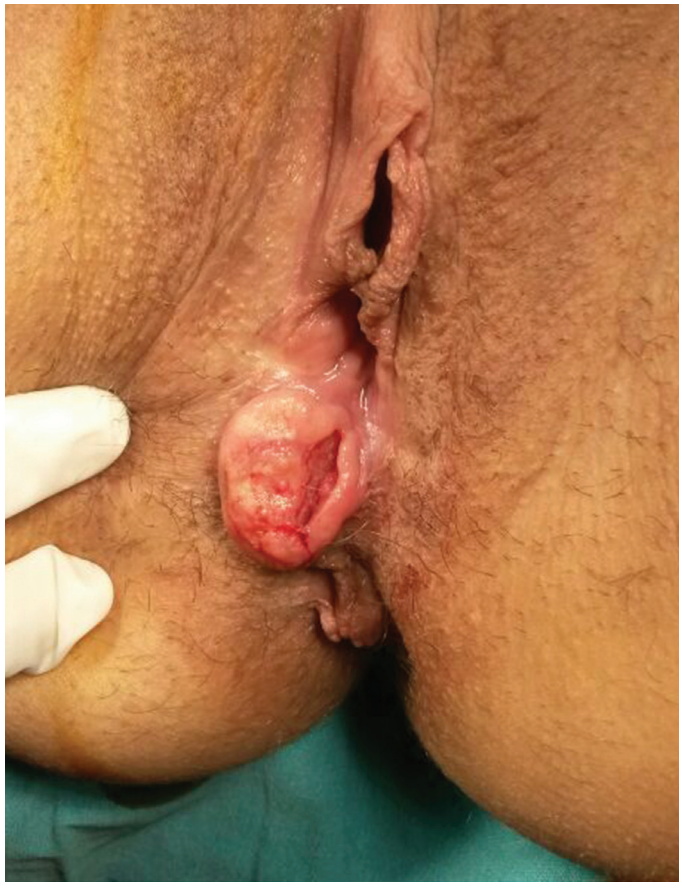

Figure 1. A 2x3-cm ulcerated fragile, firm, nodular lesion with irregular boundaries on the right labium majus with focal central hemorrhage

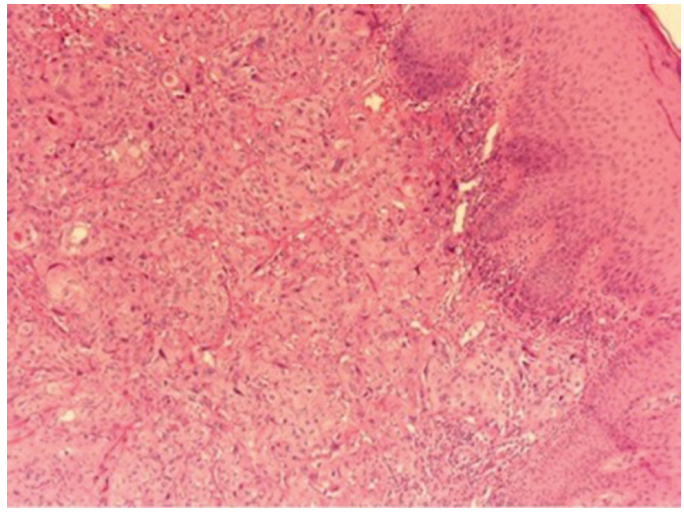

Figure 2. Infiltrating tumor nests consisting of atypical squamous cells with large abundant eosinophilic cytoplasm and a large vesicular nucleus with prominent nucleoli

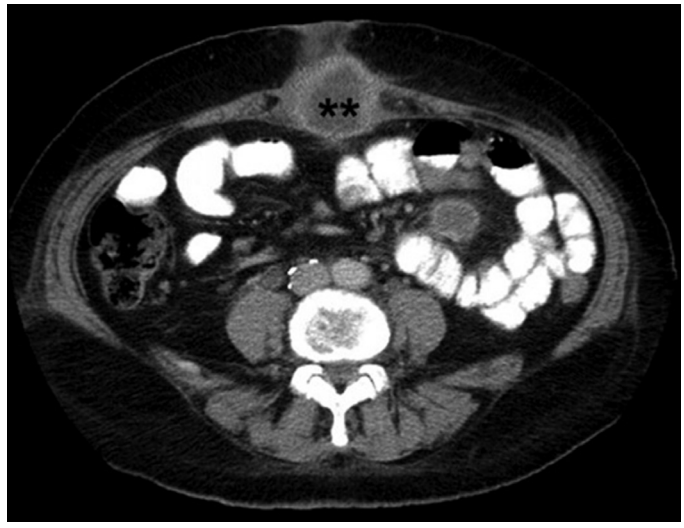

Figure 3. Computerized tomography image of metastatic umbilical mass

Table 1. Reported cases of vulvar metastasis in cervical cancer

Patient no.	Year	First author	Numberof patients	Treatment taken	Appearance of lesions after therapy	Sites of metastasis
1	2005	Srivastava[4]	1	Surgery + RT	3.5 years	Incisional skin metastasis, vulvar metastasis
2	2010	Grabiec[6]	1	Surgery + RT	No data available	Vulvar and perineal metastasis
3	2010	Deka[2]	1	CRT	6 years	Vulvar metastasis
4	2011	Kim[1]	1	CRT	2 years	Vulvar metastasis
5	2013	Richmond[7]	1	Surgery + CRT	4.5 years	Vulvar metastasis

RT: Radiotherapy, CRT: Chemotherapy

Discussion

Cutaneous metastasis usually originates from cancers of the breast, lung, ovary, colon, and rarely from the cervix. Cervical carcinoma metastases frequently occur in the vulva and anterior abdominal wall or scalp, extremities, and the umbilical surgical scar can be affected, albeit rarely[1]. Invasive interventions, including paracentesis, laparoscopy, and laparotomy can also play a role in metastases of the cervix[2]. In addition, cutaneous metastases have an incidence of 0.8% in treated cervical cancers[3]. Adenocarcinoma and undifferentiated carcinoma of the cervix are the primary histopathologic types that contribute to cutaneous metastasis. However, there is no correlation between its prevalence and clinical stages[4].

Cervical carcinoma can spread either locally or through lymphatic vessels. The lymphatic route usually follows pelvic, paraaortic and/or supraclavicular nodes, respectively. Cervical lymphatics are drained through pre-ureteral, post-ureteral, and uterosacral nodes, but these routes cannot clarify vulvar involvement. Vaginal-vulvar pathways and hematogenous invasion could be the possible routes of vulvar and umbilical incisional scar invasions, respectively. These theories have not been proven by either histologic or imaging methods. Tumor invasion to pelvic organs and the vulva can be explained by the close anatomic relationship[5]. Patients with cervical cancer metastasis can present with different symptoms. In our cases, painless skin lesions and severe abdominal pain localized in the umbilicus were the first signs of metastases during follow-up. It is very rare to detect cutaneous vulvar metastasis originating from cervical cancer before between 3.5 and 6 years after surgery[2,4]. However, this is the earliest vulvar metastasis (in the 5th month of primary treatment) of cervical cancer in the English literature. A review of the relevant literature concerning vulvar metastasis is summarized in Table 1. In contrary, there are some reports regarding early umbilical recurrence of cervical cancer in the 4th, 5th, and 6th months of primary treatment[2,8,9]. There are approximately 17 reports regarding umbilical metastasis of cervical cancer in the literature[2,10-14].

Cutaneous metastasis is considered as a hazardous condition; the mean life span is around 9 months. Regarding one study that included 1190 patients with cervical carcinoma, the incidence skin metastasis was 1.3%, which increased with advanced clinical stage as follows: 0.8% in stage 1, 1.2% in stages 2 and 3, and increasing to 4.8% in stage 4. The presence of such metastasis is associated with a high mortality rate within 2 years, regardless of the treatment procedure[15]. Therefore, the crucial prognostic factor depends on the period between the primary malignancy and cutaneous metastasis. In other words, earlier metastasis means poorer prognosis. Survival was 11 months in both of our cases, most probably due to the short recurrence period (5th month) following the primary surgical treatment.

Palliative surgery, chemotherapy, radiation therapy alone and/or in combination with cisplatin-based chemotherapy are well-known treatment modalities in managing advanced recurrent disease[16]. Based on that, we applied platinum-based chemotherapy in both cases and concurrent radiotherapy after detecting vulvar and umbilical scar extensions, respectively. Nevertheless, there was no response and we administered an alternative protocol (gemcitabine and bevacizumab) in both cases, which may have prolonged survival to up to approximately one year.

In conclusion, cervical cancer rarely leads to vulvar and umbilical incisional scar metastasis, which can be accepted as a poor prognostic factor accompanied with short life span. Physicians should always keep in mind the likelihood of recurrence at these locations during follow-up in cases of cervical cancer. To the best of our knowledge, our first case is the earliest vulvar recurrence and our second case is one of the earliest recurrences of cervical carcinoma in the English literature following appropriate surgical and medical management.

Ethics

Informed Consent: Consent forms were filled out by our two patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.M., Ö.B., Design: Z.B., Data Collection or Processing: A.M., S.S., Analysis or Interpretation: M.G.U., H.Ç.Ö., Literature Search: H.Ç.Ö., S.S., Writing: H.Ç.Ö., M.G.U.

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

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